ABSTRACT

Title of Document: **INTERVENTION STRATEGIES TO REDUCE FOODBORNE PATHOGENS IN POULTRY DURING GROW-OUT AND PROCESSING**

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Several foodborne pathogens like *Salmonella spp.*, *Campylobacter jejuni* and *Clostridium perfringens* can occasionally be traced to poultry sources. The development of intervention strategies that are applicable to different stages of poultry production can help lessen the level of these pathogens in poultry by-products and hence, reduce the incidence of poultry-borne food poisoning. In the present study, the efficacy of Poultry Litter Treatment® in reducing *Clostridium perfringens* counts in poultry litter was investigated. The effect of windrow-composting in reducing microbial load in poultry litter was also studied. In addition, a study of bacterial profiles in a poultry processing line was conducted. Finally, the efficacies of two online reprocessing antimicrobials in reducing bacterial pathogen load were compared.

INTERVENTION STRATEGIES TO REDUCE FOODBORNE PATHOGENS IN POULTRY DURING GROW-OUT AND PROCESSING

By

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Chapter 1: Introduction

1.1 Common Foodborne Pathogens in Poultry

Escherichia coli is part of the normal bacterial flora of warm blooded animals and humans. Although generally considered as commensal organisms, enteropathogenic strains of *E. coli* have been reported. Currently there are six different diarrheic groups of *E. coli* namely: Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enterohemorrhagic (EHEC), Enteroaggregative (EAEC), and Diffusely adherent (DAEC) (Fratamico *et al*, 2002). Avian pathogenic *E. coli* (APEC), found in intestines of healthy birds are mainly EPEC and ETEC (Kariuki *et al*, 2002). In the study by Kariuki *et al* (2002), 32.5% of fecal swabs taken from apparently healthy chickens and enriched for *E. coli* detection were positive for hybridization with *eae* gene of EPEC and 13.3% were positive for hybridization with *lt, st*1 and *st*2 gene of ETEC. However, according to Fratamico *et al* (2002), the EPEC serotypes found in animals are not usually associated with human infection. Asymptomatic humans are mainly the reservoirs of these EPEC while foods that are served cold are the main source of ETEC outbreaks (Fratamico *et al*, 2002). Because of the severity of diseases (hemorrhagic colitis and hemolytic-uremic syndrome) that EHEC serotype O157:H7 cause, special attention has been given to this particular serotype in food safety studies. EHEC have a characteristic of being able to produce different types of Shiga toxins (Meng *et al,* 2001). Serotype O157:H7 is the predominant cause of EHEC-associated diseases in humans in the

United States, Canada, the United Kingdom, and Japan (Meng *et al,* 2001). Undercooked ground beef (33.1%) remains the main vehicle for O157:H7 outbreaks (Meng *et al,* 2001). The prevalence of Shiga toxin producing *E. coli* in broilers is very low or absent (Beutin *et al,* 1993; Kobayashi *et al,* 2002). In the 1994-1996 Nationwide Broiler Chicken Microbiological Baseline Data Collection Program results, none of the 1,297 broilers tested was positive for *E. coli* O157:H7 (FSIS, 1996a). *E. coli* detection and enumeration is generally used as an indicator of recent fecal contamination or unsanitary food processing (Feng *et al*, 1998).

Salmonella spp. are facultative anaerobic, non-lactose fermenting members of the family Enterobacteriaceae. Because of its characteristic resistance and uncanny ability to adapt in extreme environmental conditions (low pH, high CO_2, high temperature, high salt concentration), *Salmonella spp.* poses a great concern in food safety (D'Aoust *et al,* 2001). Salmonella serotype is based on capsular, flagellar, and envelop antigens (Gray and Fedorka-Cray, 2002). According to the 2005 Salmonella Annual Summary of the Center for Disease Control (CDC, 2007), the five most commonly reported serotypes from human cases are Typhimurium (19.3%), Enteritidis (18.6%), Newport (9.1%), Heidelberg (5.3%), and Javiana (3.7%). Foodborne salmonella cases are most commonly associated with chicken consumption (Gray and Fedorka-Cray, 2002). This is due to the asymptomatic intestinal carriage in chickens which would ultimately lead to contamination of carcasses during slaughter (Gast, 2003a). However, it should be noted that it is not the host-adapted serotypes (*S. pullorum* and *S. gallinarum*) that cause foodborne outbreaks but the paratyphoid ones (Gray and Fedorka-Cray, 2002). According to the

serotype profiling study on meat and poultry products by the United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) for 2006 (FSIS, 2007b), the five most commonly reported serotypes from broiler sources are Kentucky (48.97%), Enteritidis (13.66%), Heidelberg (11.34%), Typhimurium (8.08%), and serotype 4,5,12:i:- (4.30%). *Salmonella spp.* possess three virulence factor toxins: endotoxin which causes fever, heat labile enterotoxin which causes secretory diarrhea, and heat stable cytotoxin which causes protein synthesis inhibition and subsequent epithelial cell damage (Gast, 2003b). *Salmonella* infection can cause four possible disease patterns namely: gastroenteritis, enteric fever, bacteremia with or without focal extraintestinal infection, and asymptomatic carrier (Gray and Fedorka-Cray, 2002).

Campylobacter jejuni subsp. *jejuni* are gram negative, curved rod bacteria with polar flagella often found in poultry. Avians are the most common host species for *Campylobacter* because of the high body temperature of birds (Keener *et al*, 2004). Campylobacteriosis is the most common foodborne bacterial illness in the U.S. accounting for an estimated 2.5 million cases annually (Mead *et al*, 1999). Although *Campylobacter spp.* are known to be susceptible to low pH, the infectious dose appears to be <1000 bacteria, considering it will be passing through gastric acids (Nachamkin, 2001). Unlike *Salmonella spp.*, *Campylobacter spp.* is typed using the Penner HS (heat stable) serotyping scheme which is determined by a capsular polysacharride (Nachamkin, 2001). *Campylobacter jejuni*, *C. coli,* and *C. lari* account for more than 99% of the human isolate with *C. jejuni* constituting 90% (Hunt *et al,* 1998). Among the different severe sequelae that are associated with *C. jejuni*

infection are Guillain-Barŕe syndrome (neurologic syndrome), Reiter's syndrome (sterile reactive arthritis), Miller Fisher syndrome, septic arthritis, osteomyelitis, and chronic recurrent diarrhea (Altekruse and Swerdlow, 2002; Keener *et al*, 2004). Unlike *Salmonella*, there is no standard subtyping scheme for *Campylobacter*. Most sporadic outbreaks peaking in summer months are associated with consumption of undercooked poultry or other foods that were cross-contaminated by raw poultry (Alterkruse and Swerdlow, 2002). Among the many virulence factors of *Campylobacter* are mucous colonization, flagellar attachment, iron acquisition, host cell invasion, and toxin production (Alterkruse and Swerdlow, 2002).

Clostridium perfringens are gram positive, spore-forming, anaerobic bacteria. According to Mead *et al* (1999), the estimated total cases of foodborne *Clostridium perfringens* intoxication are 250,000 annually while causing only about 41 hospitalizations. The likely explanation for this minimal hospitalization is localized damage to villus tip cells caused by the organism and the consequently normal epithelial turnover caused by diarrhea (*Labbe* and *Juneja*, 2002; McClean, 2001). Because of the mild and indistinguishing characteristic symptom of *Clostridium perfringens* type A food poisoning, most cases are not recognized and reported (McClane, 2001). Another likely explanation is that the infectious dose of *Clostridium perfringens* is very high (about 10^6 to 10^7 vegetative cells per gram of food) because of its susceptibility to gastric acid (McClane, 2001). However, *Clostridium perfringens* incidence in grow-out farms and in processed poultry remains high (Craven, 2001; Craven *et al*, 2001). Craven *et al* (2001) isolated *Cl. perfringens* in 94% of fecal samples and in 81% of post-chill carcass samples from

poultry flocks tested. Of particular concern in poultry with respect to foodborne *Cl. perfringens* poisoning are large roaster broilers and turkeys because of the difficulty of attaining high internal temperatures during cooking (McClane, 2001). Because of the relative heat tolerance of *Clostridium perfringens* vegetative cells, the heat resistance of its spores, and its ability to rapidly multiply (doubling in less than 10 minutes), temperature abuse during improper holding of foods is a major contributor to *Clostridium perfringens* type A poisoning (McClane, 2001). The toxin responsible for the characteristic symptom of *Cl. perfringens* type A poisoning is CPE (*Clostridium perfringens* enterotoxin). One unique characteristic of *Clostridium perfringens* intoxication is that the enterotoxin is released along with spores during sporangial autolysis (*Labbe* and *Juneja*, 2002). Another major virulence factor of *Clostridium perfringens* is the alpha toxin which is present in all toxin types (A-E). The alpha toxin is actually a phospholipase C which breaks down the lecithin in the cell membrane producing tissue breakdown (*Labbe* and *Juneja*, 2002).

1.2 Hazard Analysis and Critical Control Points

In 1996, the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) published the final rule of Pathogen Reduction: the Hazard Analysis and Critical Control Points (HACCP) System. This new regulation required all slaughter and processing plants to adopt HACCP as a system of process control, follow a Standard Operating Procedure (SOP) for sanitation, conduct daily microbial testing for generic *E. coli* to verify process control, and set pathogen reduction performance standards for generic *E. coli* and *Salmonella spp.* (FSIS, 1996b). HACCP is a system where processing establishments identify the food safety hazards,

institute controls to prevent those hazards, monitor the performance of those controls, and maintain records for assessment (FSIS, 1996b). HACCP has seven guiding principles namely: hazard analysis, critical control points (CCP) identification, establishment of critical limits, monitoring procedures, corrective actions, recordkeeping, and verification procedures (FSIS, 1996b). Hazard analysis is the identification of the food safety hazard on each process control and the preventive measures to control such hazards (FSIS, 1996b). A critical control point is a procedure or step where control can be applied and a food safety hazard can be prevented, eliminated, or reduced to an acceptable level (FSIS, 1996b). Critical limit is the maximum or minimum value which a CCP must meet to prevent a food safety hazard (FSIS, 1996b). Monitoring involves whether the critical limits are being met, and corrective actions refer to measures taken if there is a deviation from the critical limits (FSIS, 1996b). Proper record keeping and periodic verification of the HACCP system are used to determine whether the system complies with the HACCP plan (FSIS, 1996b). In addition to mandating the HACCP system, the FSIS also set up pathogen reduction performance standards for generic *E. coli* and *Salmonella spp.* based on the baseline study done by the Nationwide Broiler Chicken Microbiological Baseline Data Collection Program from July 1994 through June 1996 on raw post-chill carcass rinses (FSIS, 1996a; FSIS, 1996b). The 80th and 98th percentile of generic *E. coli* levels seen in broilers were 80 and 1100 cfu/ml, respectively (FSIS, 1996b). These results rounded to the closest power of 10 (100 and 1000) were adopted as the minimum acceptable and marginally acceptable limit, respectively (FSIS, 1996b). The *Salmonella spp.* prevalence result was 20% (FSIS, 1996b). A

more current baseline study done by the Nationwide Young Chicken Microbiological Baseline Data Collection Program from November 1999 through October 2000 showed lower baseline levels. The 80th and 98th percentile of generic *E. coli* levels seen in broilers in this study were 32 and 390 cfu/ml, respectively (FSIS, 2005). *Salmonella spp.* prevalence, likewise, was lower at 8.7% (FSIS, 2005). Another baseline study is currently being done (FSIS, 2007a) which aims to produce baseline for *Salmonella spp.* and *Campylobacter spp.* counts. According to the baseline study conducted by Berrang *et al* (2007) in 20 processing plants, the overall mean count for *Campylobacter spp.* in raw post-chill carcass rinse is 0.43 $\log_{10}$ cfu/ml.

1.3 Research Rationale

According to Mead *et al* (1999), the estimated annual total cases of *Salmonella spp.* and *Campylobacter spp.* foodborne illnesses are 1.4 million and 2.5 million, respectively, making up more than half of the estimated 5.2 million bacterial foodborne illness cases. *Salmonella* cases cause approximately 16,400 hospitalizations and 600 deaths, while *Campylobacter* cases cause approximately 13,200 hospitalizations and 100 deaths (Mead *et al*, 1999). According to the USDA Economic Research Services (2003), the annual economic cost of *Salmonella spp.* and *Campylobacter spp.* foodborne cases are $2.9 billion and $1.2 billion, respectively. Based on the epidemiological data reported by the U.S. Centers for Disease Control in 2006, the preliminary FoodNet data for 10 States showed that there were 6,655 *Salmonella* cases and 5,712 *Campylobacter* cases. The importance of continuous surveillance was highlighted by Zhao *et al* (2001) who conducted a survey of bacterial contamination around the Greater Washington, D.C. area on retail

raw meat. They found that 70.7% of the chicken carcasses sampled were positive for *Campylobacter spp.*, 38.7% were positive for *E. coli,* and 4.2% were positive for *Salmonella spp*. Even if the final cooking process assures the killing of these common foodborne pathogens, the results of these descriptive studies show the possible source of infection if proper cooking practices are not followed. Evidently, foodborne illnesses remain the primary concern for the general public, public health officials, and the food production industry. Employment of effective intervention strategies by applying the "multiple hurdle approach" (Russell 2007c) to reduce the level of foodborne bacterial pathogens during food production and processing is therefore critical in minimizing foodborne illnesses.

1.4 Research Objectives

The main goal of this research was to evaluate the effectiveness of various intervention strategies for pathogen reduction employed by poultry companies during different stages of production and processing. This research was focused only on the grow-out and processing aspects of a vertically integrated poultry production system. On the production side, the objective of the descriptive study was to ascertain whether *Clostridium perfringens* counts between gangrenous dermatitis positive farms were higher than those of negative farms. Results from this study could serve as a model on how a poultry disease, namely gangrenous dermatitis, can be a possible source of foodborne pathogens for humans. The objective of the PLT® study was to evaluate the efficacy of sodium bisulfate in reducing the load of *Clostridium perfringens* in poultry litter, a potential source of foodborne pathogens. The objective of the

composting-windrow study was to evaluate the ability of the windrow technique to decrease bacterial pathogen load in poultry litter that is bound to be recycled for the next grow-out cycle. On the processing side, a simple line study was carried out with two objectives in mind. First, the levels of bacterial pathogen reduction at different stages of the poultry processing line were characterized. Secondly, the baseline pathogen counts of the poultry farm involved in the present study was established to provide the farm manager with information for improving management schemes. The comparative online reprocessing antimicrobial study also carried two specific objectives. The first objective was to compare the efficacy of two antimicrobials in reducing bacterial pathogens in poultry carcasses. This data is extremely important for processing managers in deciding whether it is cost-effective to upgrade or change the antimicrobials currently being used as an online reprocessing chemical. The second objective was to compare the level of bacterial pathogens in the two evisceration lines and assess whether standards were being met.

Chapter 2: Descriptive Study of the Microbial Profile of Poultry Litter from Chronically Affected Gangrenous Dermatitis Farms

2.1 Review of Literature

2.1.1 Gangrenous Dermatitis

Gangrenous dermatitis is caused by *Clostridium septicum*, *Clostridium perfringens* type A, *Clostridium novyi, Clostridium sordelli,* and *Staphylococcus aureus* infection (*Wilder et al*, 2001). It occurs in broiler chickens at 17 days to 20 weeks of age but mostly at 4-8 weeks of age (*Wages* and *Opengart*, 2003a). Neumann *et al* (2006) reported that 23.5% of gangrenous dermatitis lesions sampled were positive for *Cl. perfringens,* 41.2% were positive for *Cl. Septicum,* and 29.4% were positive for both. Although *Clostridium perfringens* is commonly implicated as the cause of gangrenous dermatitis, there have been published case reports of *Staphylococcus*-induced (*Cervantes et al*, 1988) and *Clostridium septicum*-induced (*Willoughby et al*, 1996) cases. In an experimental *in vivo* model study, Wilder *et al* (2001) observed that *Staphylococcus aureus* and *Clostridium septicum* had either a synergistic or an additive effect. In one combination, the mortality was high but was zero when either organism was inoculated alone (synergistic). In another combination, the mortality was high, but when inoculated alone, the *Staphylococcus aureus* strain caused low mortality (additive). It is thought that gangrenous dermatitis is a consequence of immunosuppressive viral infections such as Infectious Bursal Disease (IBD), Chicken Anemia Virus (CAV), reticuloendotheliosis virus, and

Inclusion Body Hepatitis virus (IBH) (*Wages* and *Opengart*, 2003a). With CAV and avian reovirus infection, gangrenous dermatitis occurs secondary to the hemorrhagic lesions caused by blue wing disease (*Wages* and *Opengart*, 2003a). Other management related factors leading to wounds or weakened skin associated with gangrenous dermatitis are caponization, wet litter, feed outages, and overcrowding (*Wages* and *Opengart*, 2003a). In addition to these, Clark *et al* (2004) enumerated some factors that may ultimately cause hysteria and subsequent cuts from the scratches: lightning storms, longer day length, increased lighting intensity, light restriction program, and low dietary sodium. Gross lesions consist of moist, gangrenous skin; subcutaneous edema and emphysema; and skeletal muscular necrosis and hemorrhage (*Wages* and *Opengart*, 2003a). Hepatic, splenic, renal, and pulmonary lesions may be present. Since *Clostridium spp.* are very durable due to their spore forming chararcteristic, the main strategy suggested by Clark *et al* (2004) is to keep the number of *Clostridium spp.* as low as possible in order to allow faster recovery.

2.1.2 Current Litter Survey Studies

The microbiological profiles of poultry litter are an important consideration because of the constant contact of poultry with litter in non-elevated floor-type houses that are commonly used in the United States. Previous microbiological surveys and profiles of poultry litter have been conducted (Martin *et al*, 1998; Hartel *et al,* 2000; Vizzier Thaxton *et al*, 2003; Terzich *et al,* 2000; Omeira *et al*, 2006; Craven *et al*, 2001). The studies by Terzich *et al* (2000) and Vizzier Thaxton *et al* (2003) only involved total gram positive and total anaerobic counts, respectively, and not a

specific count of *Clostridium perfringens.* Omeira *et al* (2006) did specific *Clostridium perfringens* counts but their litter samples were taken from healthy farms. Craven *et al* (2001), on the other hand, did an incidence survey of *Clostridium perfringens* and found 23% of the 412 litter samples to be positive. The objective of the present study was to describe the levels of *Clostridium perfringens* in farms chronically affected with gangrenous dermatitis as compared to gangrenous dermatitis free (control) farms as a way to show how gangrenous dermatitis farms may pose a threat to food safety. This is important because several millions of *Clostridium perfringens* are required to produce typical foodborne illnesses, making species-specific enumeration studies necessary to more accurately describe the food safety implication of gangrenous dermatitis affected farms (Shahidi and Ferguson, 1971).

2.2 Materials and Methods

2.2.1 Sample Size and Collection

Ten litter samples were taken from each of eight farms chronically affected with gangrenous dermatitis as well as from three control farms (negative for gangrenous dermatitis). Samples were collected as follows: three from the side walls, four from the drinkers, and three from the center of the house. Sampling was performed by scooping a handful of litter and placing it in a sealable plastic bag. The samples were then shipped overnight for quantification to the Maryland Department of Agriculture Animal Health Laboratory in College Park, Maryland.

2.2.2 Bacterial Enumeration

Twenty-five grams of soil samples were dissolved in 225 ml PBS (Sigma-Aldrich Corp., St. Louis, MO) to make a 1:10 solution. The resulting solution was shaken for at least 15 minutes. Bacterial quantification was performed by spread plating using MacConkey agar (Becton, Dickenson and Company, Sparks, MD) for coliform count, Trypticase Soy agar (Becton, Dickenson and Company, Sparks, MD) for total aerobic count, and Shahidi-Ferguson Perfringens (SFP) agar (Becton, Dickenson and Company, Sparks, MD) for *Clostridium pefringens* count. Only three-fold 1:10 dilutions were made. The SFP agar (Shahidi and Ferguson, 1971) culture plates were sealed in a plastic bag using a plastic sealer with an AnaeroPak® (Mitsubishi Gas Chemical, Inc., New York, NY) included inside. Bacterial quantification was performed after 20-24 hours of incubation.

2.2.3 Statistical Analysis

All counts were transformed to log form. Using Statistical Analysis System/SAS (SAS Institute Inc., Cary, NC), PROC GLM (Generalized Linear Model) with nested arrangement was employed to analyze whether there was a statistical difference between *Clostridium perfringens* counts from chronically affected gangrenous dermatitis farms and normal (control) farms at $\alpha=0.05$.

2.3 Results and Discussion

A total of eight poultry houses were sampled as gangrenous dermatitis affected farms and three as gangrenous dermatitis negative (control) farms (Figure 1 and Appendix A). The bacterial load of gangrenous dermatitis affected farms was

consistently higher than that of control farms for TAC (total aerobic count), coliform counts, and *Clostridium perfringens* counts. The differences in all counts between farms nested under their farm status category (affected or control) were all statistically significant (Table 2). While the difference in TAC and coliform counts between affected and control farms were statistically insignificant, the difference in *Clostridium perfringens* counts was statistically significant.

The total aerobic counts for both affected and control farms were approximately 10^5 to 10^7 $\log_{10}$ cfu/ml. These counts coincide with the litter survey studies of Martin *et al* (1998) and Vizzier Thaxton *et al* (2003) but were less than that of the litter survey study of Terzich *et al* (2000). The difference in TAC between affected and control farms was about 0.20 $\log_{10}$ cfu/ml. This constitutes a very large decrease (2.5 million cfu/ml).

The coliform counts for all 11 houses were relatively low ranging from 0.10 to 3.88 $\log_{10}$ cfu/ml. This is consistent with previous studies (Hartel *et al*, 2000; Martin *et al,* 1998; Vizzier-Thaxton *et al,* 2003). Hartel *et al* (2000) reported that 10 out of the 20 fresh litter samples collected had levels lower than one $\log_{10}$ cfu/g. Martin *et al* (1998) likewise reported that five out of 86 litter samples collected all across Georgia had detectable coliform counts. Vizzier-Thaxton *et al* (2003) also reported average litter coliform counts in Mississippi to be one $\log_{10}$ cfu/g. In contrast, Terzich *et al* (2000)'s multi-state survey reported coliform counts ranging from 6 to 8 $\log_{10}$ cfu/g. This discrepancy cannot be explained. These varying observations may be attributed to the different geographical locations of these studies.

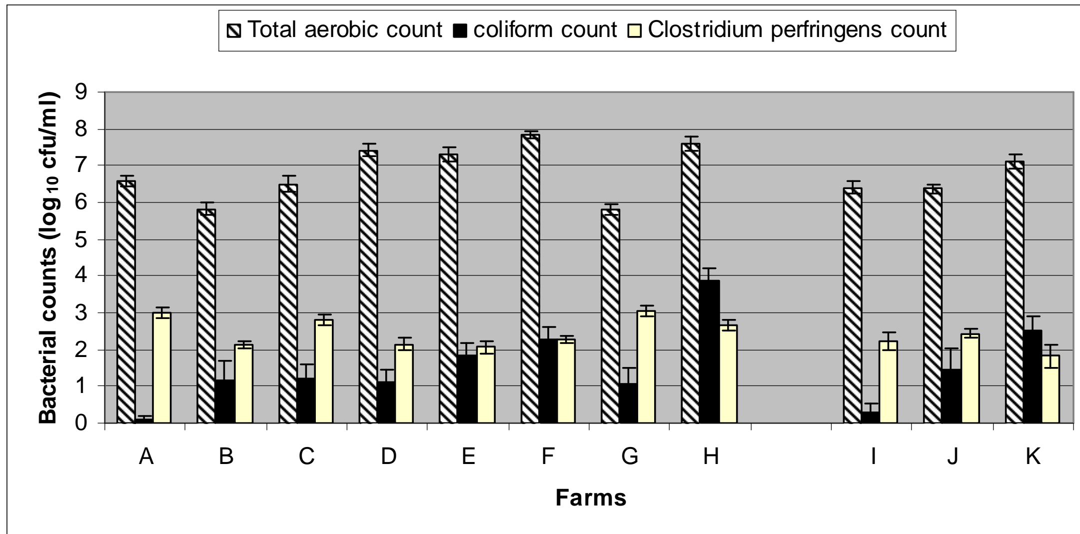

Figure 1. Average total aerobic, coliform and *Clostridium perfringens* counts in litter of gangrenous dermatitis-affected and control broiler farms
* Farms A-H represent gangrenous dermatitis-affected farms
* Farms I-K represent control farms

The *Clostridium perfringens* counts for both affected and control farms ranged from 1 to 3 $\log_{10}$ cfu/ml or 2 to 4 $\log_{10}$ cfu/g. This is higher than the Omeira *et al* (2006) study where the mean litter count for intensive broiler production was 1.05 $\log_{10}$ cfu/g. Although the difference in *Clostridium perfringens* counts between gangrenous dermatitis affected farms and control farms was statistically significant, the numerical or effective difference was only about 0.35 $\log_{10}$ cfu/ml or equivalent to about 183 cfu/ml. This result suggests that gangrenous dermatitis affected farms may not signify an effective increase in *Clostridium perfringens* counts compared to control farms. If present, *Clostridium perfringens* could possibly pose a risk to food safety along the production and processing sequence. This possibility is highlighted by the study of Craven *et al* (2003) who were able to isolate the same ribotypes of *Clostridium perfringens* in both fecal samples from grow-out farms and carcass rinses from the associated processing plant. Considering that the infectious dose of

Clostridium perfringens type A is very high (McClane, 2001) and that there are many more succeeding microbial reduction processing interventions, this slight increase in counts from gangrenous dermatitis affected farms may not entail enough added *Cl. perfringens* levels to pose an increased risk of *Cl. perfringens* type A food poisoning. Also, it should be noted that only CPE (*Cl. perfringens* enterotoxin) positive isolates can cause foodborne *Cl. perfringens* type A food poisoning (McClane, 2001).

Chapter 3: An Evaluation of Poultry Litter Treatment® (PLT®) on *Clostridium spp.* Recovery from Poultry Litter

3.1 Review of Literature

3.1.1 Clostridial Diseases in Poultry

Clostridium spp. are large, gram positive, sporeforming, anaerobic, rod-shaped bacteria. In an incidence study, Craven *et al* (2001) detected *Clostridium perfringens* in 94% of the flocks tested. Because *Clostridium perfringens* is sporogenic and ubiquitous in soil due to its anaerobic nature, the study of the effects of litter amendments to *Clostridium spp*. levels in poultry litter is important. It has also been observed that litter clean-out delays recurrence of *Clostridium* outbreaks in the poultry houses by 2-3 grow-out cycles (Bautista, personal communication). *Clostridium spp.* infections in poultry are associated with four distinct diseases namely: ulcerative enteritis, necrotic enteritis, gangrenous dermatitis, and botulism.

Ulcerative enteritis/Quail disease, caused by *Clostridium colinum*, is an acute infection mainly seen in young poultry (Wages, 2003). It occurs in chickens at 4-12 weeks of age happening often with a co-infection or stress condition and producing ambiguous signs such as watery white droppings (Wages, 2003). The spores produced by the organism in the litter would be a permanent contamination (Wages, 2003).

Necrotic enteritis is caused by alpha and beta toxins produced by *Clostridium perfringens* types A and C which are ubiquitous and part of the normal flora of healthy chickens (Wages and Opengart, 2003b). Contaminated feed and litter have

been implicated in most outbreaks (Wages and Opengart, 2003b). Ambiguous signs such as depression, anorexia, diarrhea, ruffled feathers, and sudden increase in mortality are present (Wages and Opengart, 2003b).

Botulism/Limberneck/Western duck sickness is caused by the exotoxin of *Clostridium botulinum* types A, C and E causing flaccid paralysis of the legs, wings, neck, and eyelids (Dohms, 2003). The source of infection involves maggots festering in the gut of bird carcasses, small crustraceans, and insect larvae (Dohms, 2003).

Gangrenous dermatitis is caused by *Clostridium septicum*, *Clostridium perfringens* type A, and *Staphylococcus aureus* infection. A more detailed review of literature is discussed in Chapter 2.

3.1.2 Poultry Litter Amendment

Ammonia is produced by microbial decomposition of uric acid, the principal nitrogenous waste product in avian species. Other substrates for ammonia production are organic nitrogen from avian feces and ammonium from the decomposition of spilled feeds (Shah *et al*, 2006). The interplay of high pH, ammonia production, and ureolytic bacteria causes a vicious cycle of promoting the propagation of each factor as highlighted by Blake and Hess (2001). According to Blake and Hess (2001), increasing pH increases ammonia concentration. High pH also encourages uric acid decomposition which would produce more ammonia. High pH promotes the maximal activity of uricase, the enzyme responsible for uric acid breakdown to allatoin, which subsequently will be converted to urea, and finally to ammonia by urease (Black and Hess, 2001; Ritz *et al*, 2004). Finally, high pH allows ureolytic bacteria to thrive, resulting in the use of uricase. Ammonia production is also favored in high

temperature and high moisture situations making brooder flocks more at risk (Shah *et al*, 2006).

Adding to the problem is the continuous and prolonged use of the same poultry litter in many successive grow-outs, sometimes reaching two years or even longer, resulting in ammonia build-up. The high price of litter raw materials like wood shavings, the ever decreasing land available for litter disposal, and environmental considerations are among the reasons for the recycling of poultry litter (Shah *et al*, 2006).

The detrimental effects of ammonia on poultry performance and health have been described in a few review articles (Al Homidan *et al*, 2003; Ritz e*t al*, 2004; Quarles and Caveny, 1979). Beker *et al* (2004) reported that the gain to feed ratio of broilers was reduced significantly at 60 ppm ammonia (NH_3) considering that commercial poultry are usually exposed to 50 ppm NH_3. The obvious consequence of this is decreased production and lower profits for the grower. Some authors have also associated increasing NH_3 levels to increasing susceptibility to certain infections (Moum *et al*, 1969) possibly due to stress, as well as to decreasing vaccination efficacy (Kling and Quarles, 1974; Caveny *et al*, 1981). Anderson (1964) showed higher infection rate for NDV for chickens exposed to 20 and 50 ppm ammonia than control chickens.

Aside from being detrimental to poultry health, ammonia produced in broiler houses is also an environmental and human health concern (Ritz *et al*, 2004). It helps form fine particulate matters which can aggravate asthma (Shah *et al*, 2006).

There are five types of litter amendments that control ammonia: acidifiers, alkaline materials, adsorbers, inhibitors, and microbial-enzymatic treatment. Among the currently available litter acidifiers used in the industry are: Poultry Litter Treatment/PLT® (93% $NaHSO_4$), Poultry Guard®/Acidified clay (36% H_2SO_4 soaked in clay) and Al+Clear®/Alum [$Al_2(SO_4)_3 \bullet 14H_2O$] (Shah *et al*, 2006). PLT® is made up of 93.2 % sodium hydrogen sulfate and 6.5 % sodium sulfate (Terzich *et al,* 1998b). PLT® acts by promoting ammonium retention by preventing it from being converted to ammonia, thus lowering the litter pH. Free ammonium ions are converted to ammonium sulfate, and the sodium binds with phosphate, forming sodium phosphate (Terzich *et al,* 1998b). It also acts on the ureolytic bacteria as well as the urease itself that these bacteria produce. Among the benefits given by PLT are: decreased ammonia levels, decreased fuel usage due to the reduced need for ventilation and heating during winter, improved flock performance and health, reduced bacterial populations at the farm as well as in processing plant which is mandated by HACCP, reduced beetle population, improved worker safety and health, non-hazardous unlike Al+Clear®, and increased compliance with environmental and public health regulations (Blake and Hess, 2001; Shah *et al*, 2006).

Terzich *et al* (2000) conducted a wide multi-state descriptive study to determine the prevailing levels of bacteria in poultry litter. *Clostridium perfringens* was not specifically counted, although gram positive counts were present in all states tested. Vizzier Thaxton *et al* (2003), on the other hand, conducted a litter survey in Mississippi. Although *Clostridium perfringens* was not specifically counted, general anaerobic counts were shown to be constantly high.

There have been several studies evaluating the effect of PLT® on broiler health (Terzich *et al*, 1998a; Terzich *et al*, 1998b; Nagaraj *et al*, 2007). Terzich *et al* (1998a) reported that the ascites death rate in treated litter-raised broilers (5.9%) was significantly lower than in untreated ones (31.5%). Terzich *et al* (1998b) also showed that thoracic air sac and microscopic tracheal mucosal lesion scores in treated litter-raised broilers were significantly lower than in untreated ones. On the other hand, Nagaraj *et al* (2007) showed a decreased incidence of pododermatitis in treated litter-raised broilers compared to untreated ones.

There have been studies evaluating the ability of PLT® to reduce the levels of *E. coli*, *Salmonella spp.* and *Campylobacter spp.* (Line and Bailey, 2006; Line, 2002; Payne *et al*, 2002; Pope and Cherry, 2000). However, some findings are conflicting. Line (2002) reported that PLT® significantly reduced *Campylobacter* cecal colonization frequency and levels but not *Salmonella*. On the other hand, Pope and Cherry (2000) reported that *Salmonella* on farm carcass rinse counts were lower with treatment but *Campylobacter* rinse counts were only marginally different between the treated and control groups. Payne *et al* (2002) reported that artificially inoculated litter had significantly lower *Salmonella spp.* counts in the treatment group. Line and Bailey (2006) reported that PLT® delays the intestinal tract colonization by *Campylobacter* but not *Salmonella.* These discrepancies might simply be due to differences in experimental design and highlight the need for more studies.

3.2 Materials and Methods

3.2.1 Broiler House Layout and Design

An experimental broiler facility was divided into two separate houses, east and west (Figure 2). Each house was further divided into five sampling locations longitudinally: two near the side walls, one at the center of the house, and two under the drinker lines (which are located between the center and the two side walls). These five areas were then divided cross-sectionally into three blocks (near the door, middle, and near the ventilation fan) (Figure 3). Each of the resulting cells (block X location) was then divided again cross-sectionally into treatments: PLT-treated, salt-treated, and control sub-cells organized in a randomized complete block design (Figure 4). Salt was added as a treatment because it is the most common non-commercial litter amendment used by poultry growers. PLT was applied at a rate of 100 lbs/ 1000 sq ft while salt was applied at a rate of 60 lbs per 1000 sq ft. Treatments were applied over the soil pad. Six inch lengths of rebar were driven into the corners of each sub-cell leaving about ¾ inch length protruding from the soil surface.

Three thousand chicks were placed in each house after taking the baseline counts. The birds were vaccinated via drinking water with Newcastle Disease and Infectious Bronchitis.

Experimental House Layout

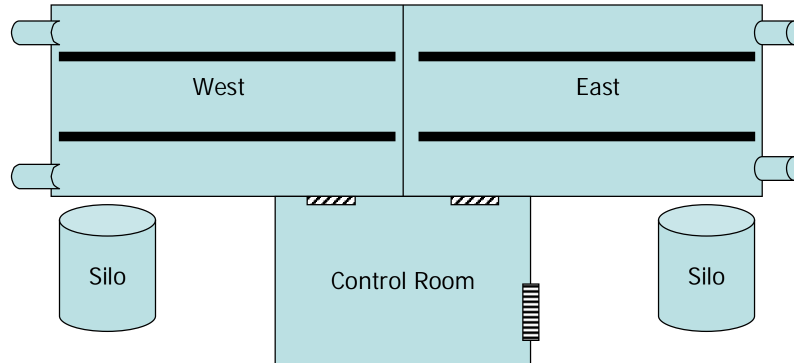

Figure 2. Layout of the litter amendment experimental houses

Door/Side	Middle/Side	Fan/Side
Door/Waterer	Middle/Waterer	Fan/Waterer
Door/Center	Middle/Center	Fan/Center
Door/Waterer	Middle/Waterer	Fan/Waterer
Door/Side	Middle/Side	Fan/Side

East House

Fan/Side	Middle/Side	Door/Side
Fan/Waterer	Middle/Waterer	Door/Waterer
Fan/Center	Middle/Center	Door/Center
Fan/Waterer	Middle/Waterer	Door/Waterer
Fan/Side	Middle/Side	Door/Side

West House

Figure 3. Compartmentalization of the litter amendment experimental houses into blocks and location (Blocks/Location)

Experimental Broiler House Layout

PLT	C	S	S	PLT	C	C	S	PLT
C	S	PLT	PLT	C	S	S	PLT	C
S	PLT	C	C	S	PLT	PLT	C	S
PLT	C	S	S	PLT	C	C	S	PLT
C	S	PLT	PLT	C	S	S	PLT	C

Study design by: Dr. Susan White, Univ. of Delaware
PLT=Poultry Litter Treatment; S=Salt; C=Control

Figure 2. Randomized complete block design of the litter amendment houses

3.2.2 Litter Sampling and Bacterial Enumeration

Each litter sample was collected by scooping a handful of litter and soil and placing it in a sealable plastic bag during pre-treatment/baseline and at post-treatment (weeks 3, 5, and 7). The samples were then transported for quantification to the Maryland Department of Agriculture Animal Health Laboratory in College Park, Maryland. Twenty-five grams of soil samples were dissolved in 225 ml PBS (Sigma-Aldrich, St. Louis, MO) to make a 1:10 solution. The resulting solution was then shaken for at least 15 minutes. Bacterial enumeration was performed by direct spread plating using SFP (Becton, Dickenson and Company, Sparks, MD) agar for *Clostridium pefringens* (Shahidi and Ferguson, 1971). Since it was shown in the pilot study that most bacterial counts are within log 4, only three 1:10 dilutions were made. The SFP agar culture plates were sealed in a plastic bag using a plastic sealer with an

AnaeroPak® (Mitsubishi Gas Company, Inc., New York, NY) included inside. Bacterial enumeration was performed after 18-24 hours of incubation.

3.2.3 Statistical Analysis

All counts were converted into log forms. Using Statistical Analysis System/SAS (SAS Institute Inc., Cary, NC), PROC GLM was employed to test differences between blocks, locations, and treatments for statistical significance in litter *Clostridium perfringens* counts during 3, 5, and 7 weeks of age as well as soil pad *Clostridium perfringens* counts during pre-treatment/baseline and during harvest using a randomized complete block design at $\alpha = 0.05$. The t-test on the difference between two treatment means was also employed at $\alpha = 0.05$ to compare PLT counts with Control counts and Salt counts with Control counts.

3.3 Results and Discussion

Without factoring blocks and locations, the comparison of treatment effect on *Clostridium perfringens* counts was inconclusive and did not show any general pattern (Appendix C, Figures 5 and 6). At week 3 of age, PLT®-treated litter showed the lowest average *Clostridium perfringens* count in the west house but almost the same as the untreated litter in the east house. The salt-treated litter showed a lower average count than the untreated litter in the west house but produced the highest average count in the east house. At week 5 of age, PLT®-treated litter resulted in the lowest average count in the east house but produced the highest average count in the west house. The salt-treated litter resulted in a lower average count than the untreated litter in the east house but produced almost the same average count in the west house.

At week 7 of age, the PLT®-treated litter showed a higher average count than the untreated litter in the east house but almost the same average count in the west house. The salt-treated litter, likewise, produced the same profile as the PLT®-treated litter. During harvest (week 7), the PLT®-treated soil pad resulted in the lowest average count in the east house but produced the highest average count in the west house. The salt-treated soil pad resulted in a higher average count than the untreated soil pad in the west but almost the same count in the east house. Using the t-test for comparison between two means, all possible two treatment combinations produced statistically insignificant differences (Appendix D).

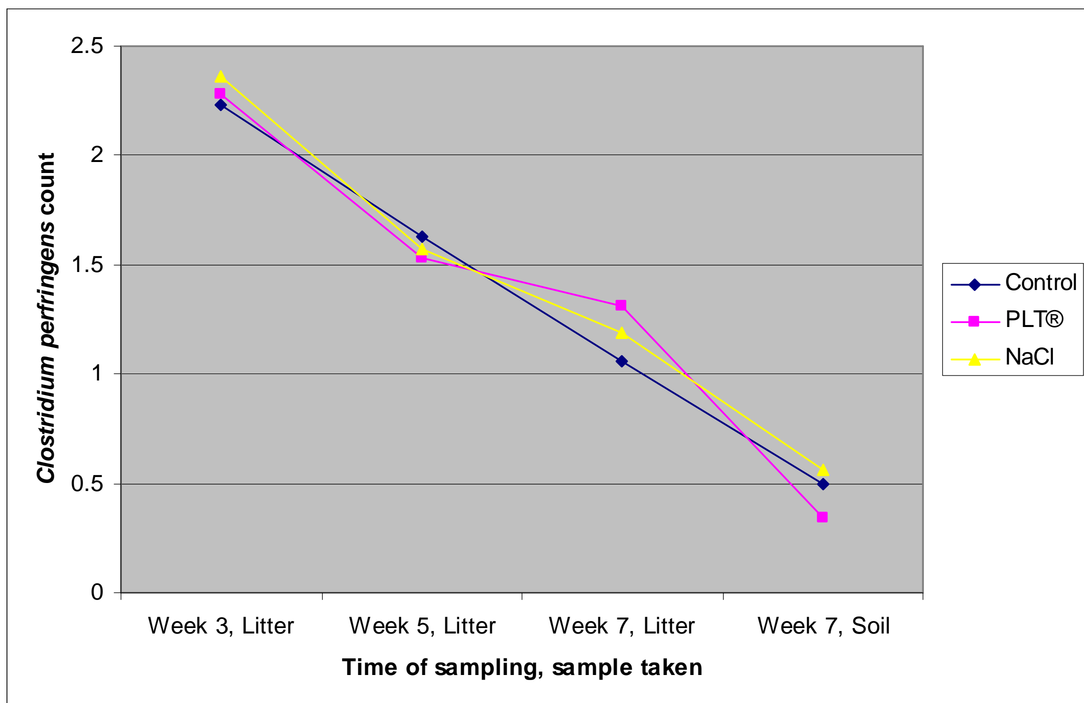

Figure 5. Average *Clostridium perfringens* counts in the east house

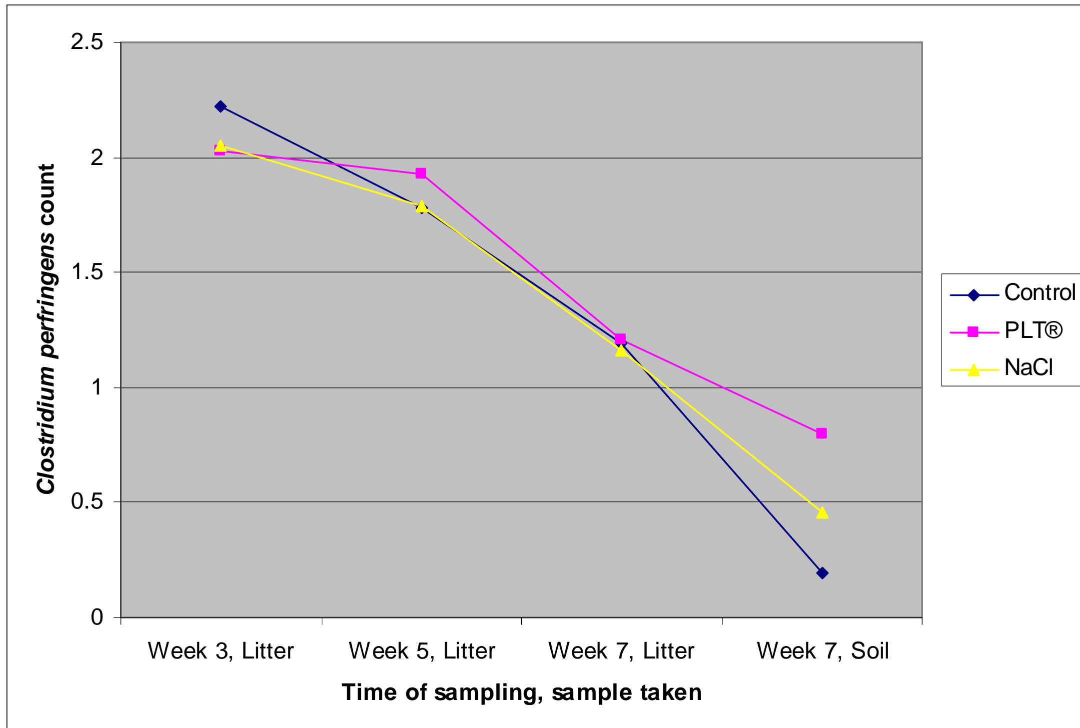

Figure 6. Average *Clostridium perfringens* levels in the west house

The results of factoring in the block and locations among the treatment in a randomized complete design using GLM are shown in Appendix E and Appendix F. The differences in the blocks and locations in the baseline soil counts for both houses were all statistically insignificant. Only the differences between blocks for both houses among the week 3 litter counts were statistically significant. In the east side, the door block samples showed the highest least square means while in the west side, the middle block showed the highest least square means. Both the differences between the blocks and locations in the east house as well as the differences between the blocks in the west house among the week 5 litter counts were statistically significant. In the east side, the door block and waterer location samples showed the highest least square means while in the west side, the fan block showed the highest least square means. The statistically significant difference in the week 7 litter counts

were the same as those of week 5. In the east side, the door block and waterer location samples showed the highest least square means while in the west side, the middle block showed the highest least square means. Both the differences between the locations and the interaction of the location and treatment in the east house as well as the differences between the treatments in the west side among the harvest soil counts were statistically significant. In the east side, the center location samples showed the highest least square means, while in the west side, the salt treatment showed the highest least square means and the PLT® treatment the lowest least square means. These results show that for litter samples, blocks produced significantly different results for both houses regardless of the timepoint of sampling. This highlights the importance of using a randomized complete block design. For litter samples collected beyond week 3, the locations in the east house produced significantly different results. In both cases, the waterer location showed the highest least square means. This can be explained by the fact that dripping water from drinkers can enhance the growth of *Clostridium perfringens*. The results in the blocks and locations are in contrast with the study of Craven *et al* (2001) who reported that wall and fan swabs had the highest incidence of *Clostridium perfringens.* It should be noted, however, that enumeration patterns in litter may not necessarily follow the incidence pattern in area swabs. All the treatment differences except in the soil pad of the west side during harvest were statistically insignificant after factoring the blocks and location. In one case where the difference was statistically significant, PLT®-treated soil pads showed lower least square means than the controls (Figure 7). However, the effective difference was only 0.12 $\log_{10}$ cfu/ml or about 1.32 cfu/ml.

The prolonged five-month interval between treatment application and actual harvest could possibly be the reason for this very small difference in counts.

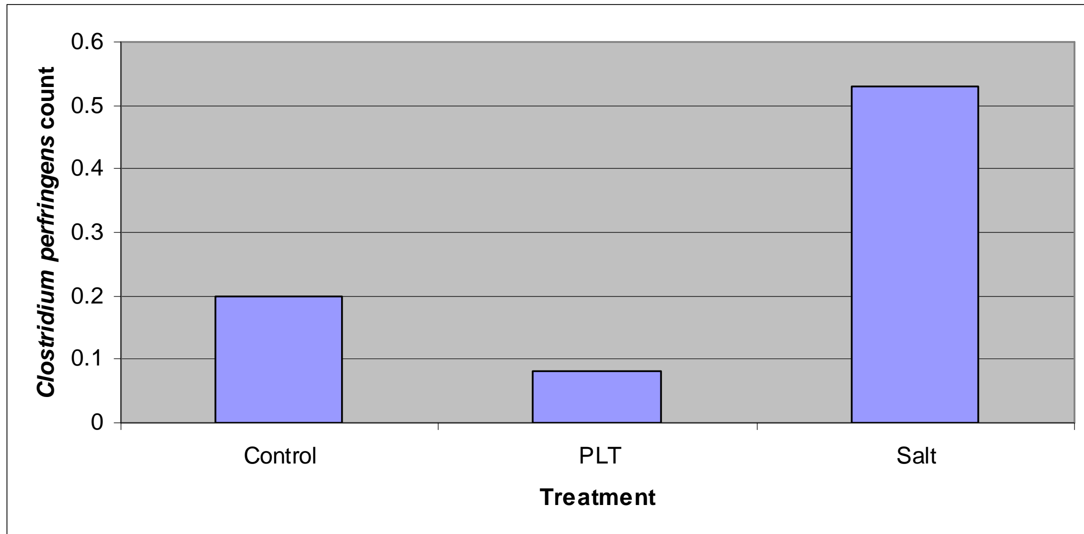

Figure 7. Least square means of *Clostridium perfringens* counts in soil during harvest at the west house

There have been several studies evaluating the effect of sodium bisulfate treatment in litter on bacterial pathogen counts mostly dealing with coliform, *E. coli, Salmonella spp.,* and *Campylobacter spp.* (Pope and Cherry, 2000; Line, 2002; Payne *et al,* 2002; Line and Bailey, 2006). Of these studies, only the Pope and Cherry (2000) study directly measured the actual litter sample bacterial load to evaluate the effect of the treatment. Line's (2002) measure of effect was cecal sample and whole carcass rinse counts while Payne *et al* (2002) evaluated the efficacy of sodium bisulfate by measuring artificially inoculated litter in a pan. Clearly, the results from the present study were not conclusive. One possible reason why this study failed is that the design was flawed. There was no compartmentalization of the different treatment groups. This would have caused the bacteria from litter of one treatment group to be carried by broilers as they roamed around the experimental houses. In

previous PLT® evaluation studies, the researchers used a study design with separate pens, chambers, or houses (Pope and Cherry, 2000; Nagaraj *et al*, 2003; Terzich *et al,* 1998a; Terzich *et al*, 1998b; Line 2002). Another major problem was the execution of the study with regards to the length of time between the application of the treatments (July 2007) and the placement of the birds (October 2007). The reason for this three month delay was the need for fixing the electrical wiring for the lights of the experimental house. One possible explanation is that the efficacy of the sodium bisulfate was eroded by neutralization with ammonia being produced by remnant ureolytic bacteria in the soil. In the Pope and Cherry (2000)'s study, the researchers evaluated the litter only up to week 2 post-treatment with chick placement almost immediately after treatment.

However, there are some valuable observations that can be taken from the present study. It was shown that *Clostridium perfringens* counts in litter samples from both houses were progressively declining over time (Figures 5 and 6). Whether this is caused by increased competition by other bacteria due to increased bacterial load of the houses as the broilers grew, or an effect of the sodium bisulfate and salt treatment is impossible to ascertain with this design as other bacterial levels were not quantified. This observation is similar to Craven *et al*'s (2001) observation where the incidence of *Clostridium perfringens* from fecal samples inside the house decreased with time. However, it should be noted that the enumeration pattern in litter may not necessarily follow the incidence pattern in fecal samples collected over the litter. In the soil pad during the harvest of broilers in both houses, where the effect of mixing of bacteria by roaming broilers was not a factor, PLT®-treated litter had lower counts

than controls and salt-treated litter. This information is still useful since cumulative bacterial load through successive litter use would eventually end up in the soil pads. The reduction of bacterial load in the soil pad would be beneficial to eliminate pathogen reservoirs when the litter is finally changed after several cycles. Even if there is a barrier (the litter) between the soil pad and the broiler, successive litter use over many grow-out cycles would lead to decreasing litter thickness. This scenario would make it more likely that broilers can dig through the thin litter and have contact with the soil pads. The different blocks at different time points for both houses produced significantly different counts which shows that block design in this study was very important.

Although HACCP is not currently mandated in the grow-out side of poultry production, intervention strategies such as litter amendments may help lessen the build-up of these foodborne pathogens in the poultry house environment and subsequently reduce the level of exposure of broilers to these pathogens.

Chapter 4: Effect of Windrow Composting on the Microbiological Profile of Poultry Litter

4.1 Review of Literature

Poultry litter in the Delmarva peninsula is currently re-used for several cycles, sometimes for as long as two years (Bautista, personal communication). Some of the reasons why poultry litter is re-used are environmental concerns and the cost of new litter materials (Lavergne *et al,* 2006). One of the other major uses of poultry litter is as crop fertilizer. Direct applications of poultry litter as organic fertilizer has some deleterious consequences such as potential spread of pathogens (Kelleher *et al*, 2002). In addition to being used as a fertilizer, poultry litter is also sometimes used as an alternative feedstuff for beef and dairy cattle (Martin *et al,* 1998; Terzich *et al,* 2000; Jeffrey *et al*, 1998). Among the alternatives in disposing poultry litter are composting, centralized anaerobic digestion, and direct combustion (Kelleher *et al*, 2002). Composting may be a cheaper alternative than litter amendment in eliminating bacterial pathogens in used litter (Macklin *et al*, 2006). Windrow pile heating or windrow composting is a method used to sanitize recycled poultry litter. Composting is not merely the piling of litter and manure to generate heat but is an active aerobic process requiring aerobic bacteria, oxygen, and a carbon source to drive the decomposition. There are several types of composts namely: static piles, passively aerated piles, and mechanically turned windrows (Brodie *et al,* undated). According to Brodie *et al* (undated), although static pile composting requires less labor, it does take a longer time than turned compost to achieve the required degradation

temperature. In fact, mechanically turned windrow compost would only take only about 5-10 days to complete (Macklin *et al,* 2007). Since poultry litter naturally would have a very high nitrogen (N) content due to uric acid and partially digested proteins, additional carbon (C) sources usually from organic material such as sawdust, pine shavings, or peanut hull have to be added in order to achieve an appropriate C:N ratio for optimum degradation (Atkinson *et al,* 1996). According to Brodie *et al* (undated), the ideal C:N compost ratio is 25:1 to 35:1. The optimum moisture content for composting is between 40% and 60% (Flory *et al,* 2006). Compost that is too dry (<40% moisture) or too wet (>60% moisture) will not reach the desired temperature of 135^0 F (Flory *et al,* 2006). Too dry compost would reduce the microbial diversity needed for efficient composting while too wet compost would pose the risk of increasing ammonia volatization for the next flock (Lavergne *et al,* 2006). The study of Lavergne *et al* (2006) pegged the ideal moisture content at 32-35%. In contrast, Brodie *et al* (undated) set the ideal moisture content much higher at 50-60%. The internal temperature of the compost must reach 135^0 to 145^0 F (Tablante *et al,* 2002). If internal temperature drops to 115^0 to 125^0 F, the compost must be turned for aeration (Tablante *et al,* 2002). According to Macklin *et al* (2007), there are three mechanisms whereby pathogens are killed during composting namely: heat, ammonia, and competition with fellow bacteria. There is a dearth of literature on specifically evaluating the efficacy of windrow pile heating in reducing the bacterial load of poultry litter. According to Wilkinson (2007), composting can control most pathogens except spores and prions. Macklin *et al* (2006) showed reduction in both aerobic and anaerobic bacterial counts after in-house static composting. Lavergne *et*

al (2006) showed that windrow composting can reduce anaerobic bacterial counts by as much as 1.26 $\log_{10}$ cfu/g. Macklin *et al* (2008) showed reduction in aerobic, anaerobic, *Salmonella, Campylobacter,* and *Clostridium perfringens* counts after in-house windrow composting. However, both windrow studies by Lavergne *et al* (2006) and Macklin *et al* (2008) did not involve turning/re-aeration. The objective of this study was to evaluate the efficacy of windrow-composting in reducing the bacterial load of poultry litter.

4.2 Materials and Methods

4.2.1 Windrow Construction and Litter Sampling

All poultry houses involved in this study had a history of gangrenous dermatitis. The compost windrows consisted of poultry litter, wood chips, and sawdust. No additional carbon source was added. All the feeders and drinkers were raised to make room for the compost windrow and tractor equipment. All the compacted and high moisture sublayer/cakes were removed using a skid steer loader. The interior of the house was pressure-washed to remove dust buildup. A skid steer loader or a tractor equipped with saw tooth paddle aerator (Brown Bear Corp., Iowa) was used to construct the windrow. The windrow was about 10 ft wide and 5 ft high, extending the whole length of the house. Depending on the size of the house and the depth of the litter, two or three columns of windrows were constructed. The compost was turned after 4.5 days, using the tractor equipped with a saw tooth paddle aerator. Ten (10) to 60 litter samples were taken before the windrow was formed and after it was spread. Sampling was performed by scooping a handful of litter and placing it in

a sealable plastic bag. The samples were then shipped overnight for quantification to the Maryland Department of Agriculture Animal Health Laboratory in College Park, Maryland.

4.2.2 Bacterial Enumeration

Twenty-five grams of litter samples were dissolved in 225 ml PBS (Sigma-Aldrich, St. Louis, MO) to make a 1:10 solution. The resulting solution was then shaken for at least 15 minutes. Bacterial quantification was performed by direct spread plating using MacConkey agar (Becton, Dickenson and Company, Sparks, MD) for coliform count, Trypticase Soy agar (Becton, Dickenson and Company, Sparks, MD) for total aerobic count, and Shahidi-Ferguson Perfringens agar (Becton, Dickenson and Company, Sparks, MD) for *Clostridium pefringens*. Only three 1:10 dilutions were made. The SFP agar (Shahidi and Ferguson, 1971) culture plates were sealed in a plastic bag using a commercial kitchen food sealer with an AnaeroPak® (Mitsubishi Gas Company, Inc., New York, NY) included inside. Quantification was performed after 20-24 hours of incubation. For the *Salmonella* isolation, 25 grams of litter sample were dissolved in 225 ml of buffered peptone water (Becton, Dickenson and Company, Sparks, MD). This solution was allowed to incubate at 37^0C for 24 hours. Then, 1 ml from this pre-enrichment solution was added to 9 ml of selective enrichment, tetrathionate broth (Becton, Dickenson and Company, Sparks, MD). The broth culture was allowed to incubate at 42^0C for 24 hours. Thereafter, 1 ml from this broth culture was transferred to a fresh 9 ml tetrathionate broth. This solution was allowed to undergo delayed enrichment-recovery for 5-7 days at room temperature. After delayed enrichment, a loopful of the broth culture was streaked onto an

XLT4/Xylose Lysine Tergitol-4 (Becton, Dickenson and Company, Sparks, MD) selective plate in a four-quadrant streaking fashion. Suspected typical colonies (black with pink periphery) were tested biochemically using TSI/Triple Sugar Iron (Becton, Dickenson and Company, Sparks, MD) and LIA/Lysine Iron agar (Becton, Dickenson and Company, Sparks, MD) tubes. All colonies producing typical *Salmonella* profile in TSI and LIA tubes were confirmed using the API20E® Enteric Identification System (BioMerieux, Inc, Hazelwood, MO).

4.2.3 Statistical Analysis

All counts were converted into log form. Using Statistical Analysis System/SAS (SAS Institute Inc., Cary, NC), the difference in mean bacterial count between pre-windrow and post-windrow compost was tested for significance using the t-test at $\alpha = 0.05$.

4.3 Results and Discussion

A total of four houses were evaluated for bacterial load reduction before and after windrow-composting. Two houses showed a statistically significant reduction in total aerobic count, coliform count, and *Salmonella spp* incidence (Appendix G, Appendix H. and Figure 8). All four houses showed a numerical reduction in *Clostridium perfringens* counts. However, only three of these reductions were statistically significant.

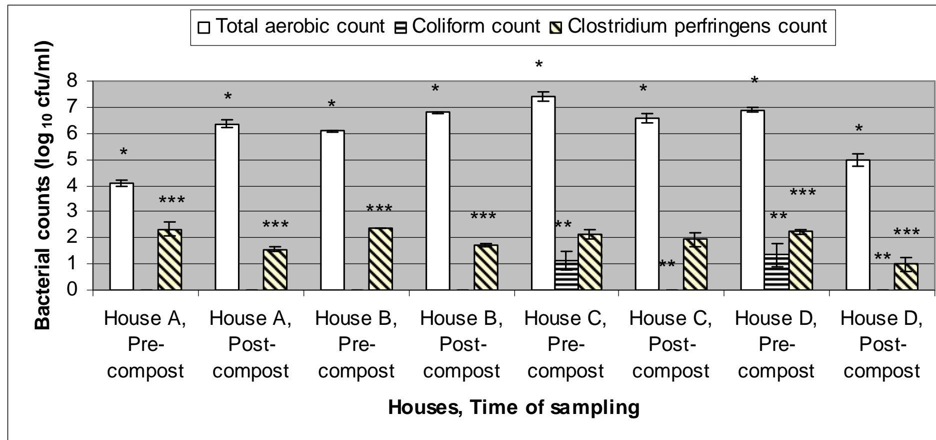

Figure 8. Average total aerobic, coliform and *Clostridium perfringens* counts in litter pre- and post-composting
* indicates that the total aerobic count reduction is statistically significant
**indicates that the coliform count reduction is statistically significant
***indicates that the *Clostridium perfringens* count reduction is statistically significant

Two of the houses showed a significant increase in TAC after windrowing. This may be attributed to the delay in counting. The other two houses showed a reduction in TAC of about 1 to 2 $\log_{10}$ cfu/ml. This is consistent with the studies of Haque and Vandepopuliere (1994) and Macklin *et al* (2006). Haque and Vandepopuliere (1994) showed a similar reduction after 22 days of composting. Macklin *et al* (2006) showed aerobic bacterial reduction of about 1 $\log_{10}$ cfu/ml both in the external and internal areas of the in-house compost pile after just two weeks.

Two houses showed undetectable coliform counts both before and after windrow formation, making comparison impossible. This is consistent with previous studies that have shown that coliform counts in litter are inherently minute (Hartel *et*

al, 2000; Martin *et al,* 1998; Vizzier-Thaxton *et al,* 2003). Of the two houses where there were pre-windrow coliform levels, both had coliform counts reduced to undetectable levels after windrowing. This is similar with the study of Haque and Vandepopuliere (1994) who reported that *E. coli* was undetectable after 22 days of composting. This is also consistent with previous studies where four out of 52 composted poultry litter samples tested yielded *E. coli* (Jeffrey *et al,* 1998). This pattern is similar to the findings of Macklin *et al* (2008) where the *E. coli* counts decreased by as much as 2.274 $\log_{10}$ cfu/g after windrow composting.

Of the four houses where there were numerical reductions in the level of *Cl. perfringens*, house D produced the largest reduction of more than 1 $\log_{10}$ cfu/ml. Macklin *et al* (2006) showed anaerobic bacterial reduction of 2 $\log_{10}$ cfu/g both in the top external and internal areas of in-house litter compost within just two weeks. This result is comparable with the house D result which had a reduction in its *Clostridium perfringens* count of about 1.25 $\log_{10}$ cfu/ml or about 2.25 $\log_{10}$ cfu/g. Macklin *et al* (2008), on the other hand, showed 8.92 $\log_{10}$ cfu/g in *Cl. perfringens* count. This large discrepancy may be explained because Macklin *et al* (2008) used artificially high amounts of inoculated *Cl. perfringens* in their study. Houses A and B, the other two houses which showed a significant reduction in *Clostridium perfringens* count, had a *Clostridium perfringens* reduction of 0.78 and 0.63 $\log_{10}$ cfu/ml or about 1.78 and 1.63 $\log_{10}$ cfu/g, respectively. This is consistent with the study of Lavergne *et al* (2006) which showed about 1.26 $\log_{10}$ cfu/g in anaerobic bacterial count. One house (house C) showed an effective but not statistically significant reduction in *Cl. perfringens* counts.

Two houses showed zero *Salmonella spp.* isolation both before and after windrow formation, making comparison impossible. Again, this result is consistent with the study of Haque and Vandepopuliere (1994) where *Salmonella spp.* was not detected both before and after windrow formation. For the two houses that showed *Salmonella spp.* isolation, one house resulted in a reduction of 30 percentage points (from 40% to 10%) while the other resulted in a reduction of 17 percentage points (from 17% to 0%). This is consistent with the study of Macklin *et al* (2008) which showed undetectable *Salmonella* counts after windrow composting.

The present study shows that windrow-composting is an effective tool for pathogen reduction in used poultry litter. Some authors like Martin *et al* (1998) contend that there is no consistent effect of composting versus non-composting on microbial numbers. However, they did not show any data to support this assumption. Reduction of litter bacterial counts is very important in integrated poultry production, especially when litter is recycled. Although HACCP is not currently mandated in the tgrow-out side of poultry production, intervention strategies such as litter composting may help lessen the build-up of these foodborne pathogens in the grow-out environment and the minimize the subsequent exposure of broilers to these pathogens. A good justification for this intervention strategy was suggested by Corrier *et al* (1999) who found that *Salmonella spp.* incidence in the crops of chickens after feed withdrawal increased instead of decreased as would normally be expected. They suggested that broilers might have ingested *Salmonella*-infected litter while being subjected to feed withdrawal. This increased incidence of *Salmonella spp.* in the crop may be a source of *Salmonella* later on during processing, especially

during evisceration due to accidental nicking or squeezing of visceral organs by an improperly aligned machine. Therefore, intervention strategies in litter management to reduce the level of pathogens have a bearing even in the later steps of live production.

Chapter 5: A Baseline Study of the Level of Bacterial Foodborne Pathogens at Different Stages of Poultry Processing

5.1 Review of Literature

Poultry processing includes all the steps involved in transforming a live bird into a ready to cook product. Wabeck (2002b) further subdivided poultry processing into the so-called "first processing" from receiving to chilling and "second processing" from chilling to shipping. This chapter will focus on the "first processing" stage of poultry processing. The basic steps in "first processing" are: feed withdrawal, catching and transport, holding, receiving, live-hanging, scalding, defeathering (picking), evisceration, washing, reprocessing, and chilling.

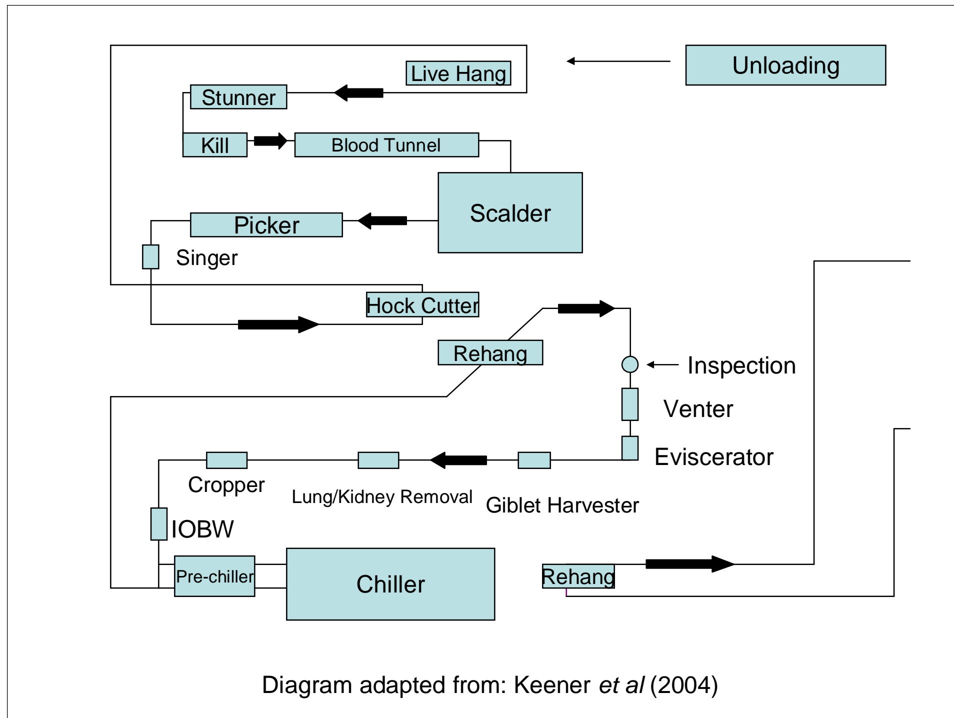

Figure 9. Basic layout of a poultry processing plant

The rationale for feed withdrawal is to reduce fecal contamination to the poultry's exterior during transport and holding and to the carcass from severed viscera during processing, facilitated by increased clearance time of feces (Keener *et al*, 2004). A carcass with filled ingesta could lead to feces coming out of the vent by slight swinging and jerking of the processing line (Russell, 2002). According to Wabeck (2002b), the optimum time for feed withdrawal is in the range of 8 to 12 hours before the start of actual processing because prolonged withdrawal time will cause increased carcass shrinkage, watery feces, and weakened intestine and gall bladder tensile strength which could lead to breakage during evisceration (Wabeck, 2002b; Russell, 2002). However, in studies by Ramirez *et al* (1997) and Corrier *et al* (1999), feed withdrawal even increased the isolation rate of *Salmonella* in the crop compared to the isolation rate before feed withdrawal. The explanation given by

Corrier *et al* (1999) was that feed deprivation could force broilers to eat *Salmonella* contaminated litter. In a similar study by Byrd *et al* (1998), feed withdrawal also increased the isolation rate of *Campylobacter* in the crop as compared to the isolation rate before feed withdrawal. Although, Northcutt *et al* (2003a) reported that even though *Campylobacter* counts may increase in the pre-chill carcass when subjected to 12 hours feed withdrawal, counts after chilling are not affected by feed withdrawal. One intervention strategy suggested in the study by Hinton *et al* (2000) was feeding a glucose-based cocktail supplement during the feed withdrawal process which resulted in fewer *Salmonella* recovery due to competitive exclusion by lactic acid bacteria.

There are currently four types of live-hauling systems employed by poultry integrators in the United States namely: steel compartmentalized cages, sliding drawer system, plastic or wooden coops, and mechanized harvester (Wabeck, 2002b). Mechanized harvesting seems to be promising as research has shown that it produces less downgrade defects than other systems (Wabeck, 2002b). Transportation, being stressful in nature, causes reduced intestinal immunity and hence, allows increased colonization of intestinal bacteria (Keener *et al*, 2004). This was shown in an experiment by Line *et al* (1997) who reported that *Salmonella* colonization rate after artificial inoculation is five-fold higher in the ceca after transport than before transport. In a study by Buhr *et al* (2000), pre-scalding carcasses hauled in solid floorings had significantly higher counts of coliform and *E. coli* compared to those hauled in wire mesh floorings. However, there was no significant difference in coliform and *E. coli* counts between the two transport floorings if the carcasses were taken after picking.

Although the studies of Northcutt *et al* (2003a) and Buhr *et al* (2000) indicate that increased bacterial load upstream of the processing line will be offset by the successive later steps such as the chiller, it is still important to make an effort to reduce bacterial load in the initial stages. This is because excessively dirty birds coming to the processing plant might overwhelm some processing equipment such as the scalding and chilling tanks and cause cross contamination downstream (Russell, 2002).

Poultry-laden trucks wait an average of 5 hours in large holding sheds before being unloaded (Wabeck, 2002b). The major consideration in the holding stage is not contamination but losses due to heat stress. The time of receiving coincides with the end of feed withdrawal and the start of actual processing. The birds are then hanged by their shackles at the start the actual processing line. Red lighting, bars for rubbing against the breast, and a minimum of about one minute interval from shackling to stunning are facilitated in order to calm the poultry prior to slaughter (Wabeck, 2002b).

The purpose of stunning is to facilitate optimum killing-bleeding and feather release. There are two methods of stunning: electrical and gas/chemical (Heath *et al*, 1994). According to the study done by Heath *et al* (1992), 85% of the 279 processing plants surveyed all over the U.S. use electrical stunning while the rest operate without stunning or use gas. The gas system is generally used outside the United States. Stunning beyond 35 volts, 0.3 amps for 8 seconds may result in broken clavicles, pericardial sac bleeding, and ruptured blood vessels in the breast muscles (Wabeck,

2002b). An interval of about 15 seconds is needed from stunning to killing to allow slight relaxation of the muscle (Wabeck, 2002b).

The two methods of slaughter are either manual (as in Kosher) or mechanical. According to Wabeck (2002b), the optimal way of mechanical slaughtering is by cutting only one side of the neck to avoid severing the trachea but still being able to cut both the jugular vein and carotid artery. The rationale for avoiding the trachea is to ensure that the bird is still breathing, thereby enabling proper bleeding (Wabeck, 2002b). The carcass is then allowed to bleed by a minimum of one minute to ensure that birds are no longer breathing before entering the scalding tank. Other than for animal welfare purposes, the other reasons for avoiding the trachea are to prevent contamination of the air sacs which happens if the birds are still breathing in the scalder tank and to avoid blood from adding to the biological oxygen demand (BOD) of the scald water draining to the waste system (Wabeck, 2002b).

The purpose of the scalder is to aid the defeathering process by increasing the feather density and friction with the picker fingers (Wabeck, 2002b), by breaking down the proteins that hold the feathers in place (Bennett, 2006), and by opening the feather follicles (Keener *et al*, 2004). The scalding stage is a crucial step for cross contamination because the opened feather follicles only close during the chilling process, trapping pathogens in contaminated tank water within the feather follicles (Keener *et al*, 2004). Because of the potential for cross contamination, several intervention strategies have been implemented to reduce this risk. Among these strategies are the use of a surge-agitation, counter-current tank, multi-stage tanks; a pre-scald brush; a post-scald rinse; an overflow of one quart fresh water per bird;

monitoring scald water pH; and keeping the water temperature to optimum levels (Wabeck, 2002b; Russell, 2002; Bennett, 2006). There are two methods of scalding: immersion and steam-spraying (Bennett, 2006). There are also two types of scalding based on the desired product: soft and hard scald (Wabeck, 2002b). A hard scald employs higher temperature (59-64^{0}C) but shorter time (30-75 seconds) while a soft scald employs lower temperature (51-54^{0}C) but longer time (90-120 seconds) (Bennett, 2006). In a study of 3-tank, counterflow scalder, Cason *et al* (2000) were able to show decreasing coliform and *E. coli* counts in scald water with each succeeding tank. Even the percentage of *Salmonella* isolation rate decreased in tank 3 compared to the two previous tanks. In the comparative study between a counter-current scalder (James *et al,* 1992b) and a conventional scalder (James *et al,* 1992a), aerobic plate counts and *Salmonella* isolation rates from carcass rinses taken from the counter-current-modified plant were significantly less than those from the conventional baseline plant throughout the processing steps. In a comparative study between a conventional multi-pass, one tank and a single pass, 3-tank, counter current with a pre-scald rinse and post-scald washer by Waldroup *et al* (1993), total aerobic, coliform, and *E. coli* counts as well as *Salmonella* incidence from carcass drips at post-scald were all significantly lower in the modified 3-tank, counter-current tank than in the conventional one. However, there was no significant difference in the microbiological profile of carcass rinses between the two scalder types at the level of post-evisceration and post-chill. In the comparative study of Cason *et al* (1999) between a two-pass, single tank and a two-pass, three tank scalder, there was no difference in aerobic bacterial counts in carcass rinses. However, it should be noted

that the carcass samples were taken after the defeathering stage, making it impossible to accurately conclude that the two scalder types had the same performance. In a study by Berrang and Dickens (2000), counts of total aerobic, coliform, *E. coli,* and *Campylobacter* from carcass rinses all decreased significantly after scalding by 1.8, 2.1, 2.2 and 2.9 log_{10} cfu/ml, respectively. This is in agreement with the study by Izat *et al* (1988) where all three processing plants had $\geq$ 1.84 log_{10} cfu/1000 cm^2 reduction in *Campylobacter* counts.

The actual defeathering process is accomplished by short, firm rubber fingers with rippled surfaces called roughers or pickers (Wabeck, 2002b). The process is refined further by passing through pinning (to manually remove the pin feathers), singeing (to burn hair feathers) and rinse-washer (to wash off blood, feathers, and loose epidermis) (Wabeck, 2002b). This stage of poultry processing is a critical control point for cross contamination because the rubber fingers may transfer pathogens to previously uncontaminated carcasses (Keener *et al*, 2004). In fact, in the study of Berrang and Dickens (2000), coliform, *E. coli* and *Campylobacter* counts all increased after the defeathering stage by 0.5, 0.7 and 1.9 log_{10} cfu/ml, respectively. This is in congruence with the study of Izat *et al* (1988) who found an increase in *Campylobacter* counts in three plants tested. Some of the control measures done to address this problem are continuous rinsing of carcass and picking equipment and use of 18-30 ppm chlorine rinse (Bennett, 2006). Head removal and hock cutting are done just after the post-picker bird washer (Wabeck, 2002b).

Upon cutting the hock joint, carcasses drop to the so-called “rehang belt” where they are led out of the slaughtering line and into the eviscerating line to be

manually rehanged (Wabeck, 2002b). It should be noted that automated rehang is also available and is recommended over manual rehang because it reduces external surface cross contamination (Bennett, 2006).

Although the term "eviscerate" may only refer to the scooping action by a spoon or finger-type apparatus to withdraw the viscera, there are several different processes that happen before and after this, constituting the whole "evisceration" process. Among them are preen gland removal, venting, opening cut, presenting, post-mortem inspection, trimming, reprocessing, salvage, giblet harvesting, lung removal, crop removal, neck removal, and final inspection (Wabeck, 2002b). The ultimate benefits of the feed withdrawal process are linked to the possible accidental nicking of intestines during the evisceration step (Bennett, 2006). Russell (2002) pointed out that maladjustment of the venter, opener, and eviscerator may cause cuts in the intestine leading to fecal contamination of the carcasses. The risk of contamination of carcasses is greater during evisceration because it involves manipulation of the intestinal tract that naturally harbors pathogenic bacteria. Among the control practices that Bennett (2006) has recommended are whole carcass rinse with 20 ppm chlorine, harvesting relatively uniform size birds, enforcing proper employee hygiene, extracting crops towards the direction of the head, and washing equipment. In a comparative study between the Stork Gamco Nu-Tech Evisceration System and the conventional streamlined inspection system, Russell and Walker (1997) reported that one farm showed no change while another showed a significant reduction in aerobic plate, coliform, and *E. coli* counts from rehang to cropper stages. The Nu-Tech Evisceration System has an innovation of totally removing the viscera

from the carcass, hence eliminating the need for presenters (Russell and Walker, 1997). In the conventional streamlined inspection system, the viscera remain attached to the carcass during inspection.

The purpose of the so-called "final washer" is to remove all the remaining contaminants in the carcass such as blood, fecal materials, and tissue fragments like membranes because of the zero tolerance policy of the USDA on fecal materials for carcasses entering the chiller (Wabeck, 2002b, Rasekh *et al*, 2005). To avoid any confusion, it should be noted that the role of the final washer has changed slightly through time. Previously, its main role was to remove visible fecal contamination as the main online reprocessing tool (20 to 50 ppm chlorine). With the advent of different stronger antimicrobials, the final washer's role is more on removing the organic load to prime the efficacy of the online reprocessing spray immediately after the washer. According to Kemp *et al* (2000), the removal of serum exudates and debris by the washer preconditions the carcass surface for the chemical action of the online reprocessing antimicrobial. The risk of fecal contamination was demonstrated by Smith *et al* (2007) who showed that an artificially cecal contaminated carcass could have greater coliform, *E. coli,* and *Campylobacter* counts than controls. This was again demonstrated by Jimenez *et al* (2003) who found a significantly higher *E. coli* count in fecal contaminated carcasses after evisceration than in controls.

There are three types of carcass washers: brush washers, cabinet washers, and inside-outside bird washers (IOBW) (Keener *et al*, 2004). Among the factors affecting the efficiency of washers highlighted by Keener *et al* (2004) are number and types of washers, water temperature and pressure, nozzle type and arrangement, flow

rate, line speed, and the sanitizing agent used. Yang *et al* (1998) gave a brief description of how an IOBW works. The carcass is initially sprayed on the outside with 9 nozzles, then, the inside with one nozzle and finally, the outside again with 9 nozzles. The inside spraying is actually two parts. First, the carcass is tilted horizontally to ensure the chemicals reach the inside through the rear end. Secondly, the carcass is tilted by 60^0 which would cause the chemical solution to flush out via the neck cavity. The effectiveness of the final washer in reducing some pathogenic bacteria has been shown to be significant in some studies while minimal in others. In a study by Jimenez *et al* (2003), the enterobacteriaceae, *E. coli*, and coliform counts in fecal contaminated carcasses passing through the IOBW were decreased by 9.7%, 7.9%, and 0%, respectively. In a study by May (1974) using a spray type washer, total aerobic counts were reduced at an average of 56% from four plants using swabs taken from an area at the back of the carcass. In the study by Izat *et al* (1988), all three plants tested had reduced *Campylobacter* counts. In contrast, in a study by Bashor *et al* (2004), *Campylobacter* counts in the post-washer were merely reduced by a range of 0.26 to 0.66 $\log_{10}$ cfu/ml in the four plants studied compared to counts just before the final washer. In a study by Northcutt *et al* (2003b), coliform and *E. coli* counts were not significantly decreased by IOBW.

Currently, most plants use continuous online processing (COP) type of reprocessing, employing various antimicrobial chemical solutions in order to comply with the USDA zero tolerance policy for carcasses with visible fecal contamination entering the chiller (Russell, 2007b). A more in-depth review of literature is discussed in Chapter 6.

The main purpose of the chiller is to improve the quality and shelf life of the carcass by limiting the growth of spoilage and pathogenic bacteria (Oyarzabal, 2005). Chilling is not merely putting the carcass in a tank filled with slush ice as the USDA mandates chillers to have an overflow rate of one-half gallon per bird chilled to prevent microbial buildup (Wabeck, 2002b). The addition of 20 to 50 ppm chlorine water is required by USDA to minimize cross contamination (Keener *et al*, 2004). James *et al* (1992) was able to show that the reduction of aerobic plate and Enterobacteriaceae counts in chillers with chlorination is significantly higher than in chillers without chlorination. It is also important to maintain chiller water pH of 6 to 6.5 in order to favor the formation of hypochlorous acid over hypochlorite ion (Russell and Keener, 2007; Bennett, 2006). Like the scalding tank, immersion chillers also employ the countercurrent flow mechanism to prevent buildup of organic matter (Sanchez *et al*, 2002; Russell, 2002). Chilling is actually made up of two stages: the pre-chiller and final chiller. The purpose of the pre-chiller is to gradually lower the temperature of the carcass to prevent tough meat caused by rapid muscle shortening (Wabeck, 2002b). Pre-chillers are maintained at 50^0 to 65^0 F and consist mainly of the overflow coming from the final chiller while the final chiller is maintained at 32^0 to 34^0 F (Wabeck, 2002b). The two main types of chilling are: immersion chilling and air chilling. Air chilling is used in Europe and Canada where temperatures are maintained in a refrigerated blast room to about 20^0 F (Sanchez *et al*, 2002; Wabeck, 2002b). The main advantage of immersion chilling is faster reduction of temperature and the benefit of rinsing some bacterial load, while the main advantage of air chilling is the prevention of cross contamination that occurs in the immersion chiller

tank (Sanchez *et al*, 2002). In a study by Sanchez *et al* (2002) comparing the microbiological profile of carcasses between the two types of chillers, air chilled carcasses were found to have 6% less incidence of *Salmonella spp.* and 9.4% less incidence of *Campylobacter spp.* In the study of Berrang and Dickens (2000), total aerobic, coliform, *E. coli,* and *Campylobacter* counts decreased by 0.7, 0.3, 0.4 and 0.8 log_{10} cfu/ml, respectively after chilling.

The final intervention strategies applied to poultry carcasses during processing is the post-chill dip (Russell, 2007c) or a post-chill spray (Stopforth *et al*, 2007). These interventions may be important if samples are recontaminated in the chiller tank in underperforming plants (Stopforth *et al*, 2007). In a survey conducted by Russell (2007c), acidified sodium chlorite is the most commonly used antimicrobial (67 percent). In a study by Oyarzabal *et al* (2004), the use of acidified sodium chlorite as a post-chill antimicrobial tremendously decreased *Campylobacter* counts and prevalence and eliminated *E. coli* counts to undetectable levels in two independent experiments.

5.2 Materials and Methods

5.2.1 Sample Collection

Carcass rinse samples were taken from a poultry processing plant in Arkansas. Five samples were taken randomly from different stages of the processing line namely: receiving, post-scalder, post-picker, rehang, post-evisceration, post-washer, pre-SANOVA™, post-SANOVA™, and post-chill. All the samples taken were from the same poultry flock. In order to ensure unbiased results, the first sample was taken

after some time that the processing line had been running. The sampling took place early in the morning. Each whole carcass was placed in a bag of 100 ml buffered peptone (Becton, Dickenson and Company, Sparks, MD) and shaken manually in the so called "shake and bake" method for one minute. Fifty ml of the carcass rinse from each bag were then transferred to a sterile dilution bottle and placed in a styrofoam box containing crushed ice. Samples were held in this manner during transport. Samples were then sent to the University of Delaware Lasher Poultry Diagnostic Laboratory in Georgetown, Delaware.

5.2.2 Bacterial Enumeration.

Upon receiving the samples, serial dilutions were done up to 1:100,000. Direct spread plating on MacConkey/MAC (Becton, Dickenson and Company, Sparks, MD), Xylose Lysine Tergitol-4/XLT4 (Becton, Dickenson and Company, Sparks, MD) and mCampy-Cefex (Oyarzabal *et al*, 2005) were used to quantify coliform, *Salmonella spp*., and *Campylobacter spp*., respectively. A replicate was done for each sample. mCampy-Cefex plates were sealed using a plastic sealer along with MicroAeroPak™ (Mitsubishi Gas Company, Inc., New York, NY) which provided the microaerophilic environment that *Campylobacter spp.* requires (Oyarzabal *et al,* 2005). Plates for coliform and *Campylobacter spp*. were read after 24 hrs incubation while *Salmonella spp*. were read after 48 hrs incubation. MAC and XLT4 were incubated at 37 ^{0}C while mCampy-Cefex was incubated at 42 ^{0}C. Five representative colonies from each processing stage were taken from mCampy-Cefex plates. Their biochemical profiles were tested to categorize them into either *C. jejuni, C. coli,* or *C. lari* (Hunt *et al,* 1998).

5.2.3 Statistical Analysis

All bacterial counts of zero were replaced with 1 to allow log transformation. All statistical measures were done in $\log_{10}$ cfu/ml unit. Using Statistical Analysis System/SAS (SAS Institute Inc., Cary, NC), the differences in bacterial counts for each pair of consecutive processing points were tested for statistical significance using the t-test between two means at $\alpha=0.05$.

5.3 Results and Discussion

There have been many publications evaluating the baseline total aerobic, coliform, enterobacteriaceae. and *E. coli* levels as well as *Salmonella spp.* incidence and levels in the different stages of the poultry processing plant (Appendix I and Appendix J). A similar table for *Campylobacter spp.* counts was presented in a review article by Keener *et al* (2004). Since baseline average coliform levels and *Salmonella* isolation rates would depend on many different factors such as sampling method (carcass rinse, carcass enrichment, neck skin, back swab, sponge swab), rinse solution, neutralizing diluent, diluent volume, sample size, species sampled (chicken or turkey), sampled plant specifications (e.g. with or without OLR), country/location sampled, and sensitivity and specificity of method of microbiological enumeration or isolation; it is not possible nor appropriate to compare the resultant bacterial levels to the previous baseline study from other plants to determine whether process control is properly being met in the current plant sampled. Rather, they may serve only as a guide in evaluating the bacterial load reduction by process control. Under the HACCP final rule set in 1996, only the post-chill carcass has a minimum performance benchmark. The USDA-FSIS categorized generic *E. coli* counts into 3 classes: ≤100

cfu/ml (acceptable), >100 cfu/ml but ≤1000 cfu/ml (marginal), >1000 cfu/ml (unacceptable) (FSIS, 1996b).

Coliform counts were shown to have decreased successively except after the evisceration stage (Appendix K, Appendix L and Figure 10). The coliform levels between the receiving and the post-scald carcass rinse decreased significantly by about 0.95 $\log_{10}$ cfu/ml. This is consistent with the study of Berrang and Dickens (2000) where the coliform counts decreased by 2.1 $\log_{10}$ cfu/ml. This huge discrepancy in reduction might be due to the different pre-scalding points used between the two studies. In our study, the pre-scald point was the receiving stage, while in the Berrang and Dickens' study, the pre-scald point was the post-bleed stage. Blood could have caused an increase in the pre-scald (post-bleed) stage in the Berrang and Dickens study as it is widely known that blood is a rich source of nutrients for bacterial growth, causing the reduction to appear larger. This may also be due to the difference in scalding specification. Berrang and Dickens used soft scald (55.4^0C for 2.5 min) and a three stage counter-current tank while our study used hard scald and only a single stage counter-current tank. This discrepancy between the two studies caused by varying study design and plant specifications again highlights why baseline data from other plants from previous studies cannot be fairly compared to the data from the current plant being studied. The current hard immersion scalder employs 61^0C water with 20 to 50 ppm chlorine for 90 seconds. Since the FSIS only has performance benchmarks for post-chill carcass, we decided to compare our data with that of post-scald coliform counts of previous studies. Previous post-scald carcass rinses ranged from 1.8 to 2.9 $\log_{10}$ cfu/ml (Figure 11). This range is

considerably lower than our data of 4.53 log_{10} cfu/ml. This huge discrepancy cannot be explained as the specific parameters and practices of the current plant under study were not fully disclosed. The combined effects of high temperature and chlorine can explain why there was almost a one log_{10} cfu/ml reduction in coliform counts. Considering that the plant under study employs only a single stage tank, no pre-scald brush and no post-scald rinse, the scalder tank performance and maintenance appear to be excellent.

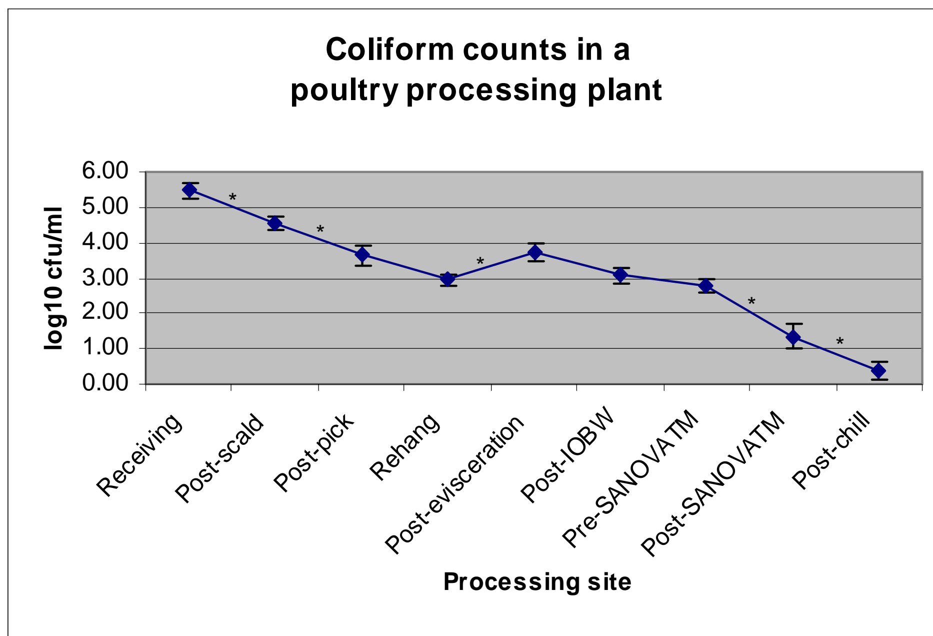

Figure 10. Line chart of average coliform counts of carcass rinses taken from different stages of a poultry processing plant
* indicates that the reduction is statistically significant

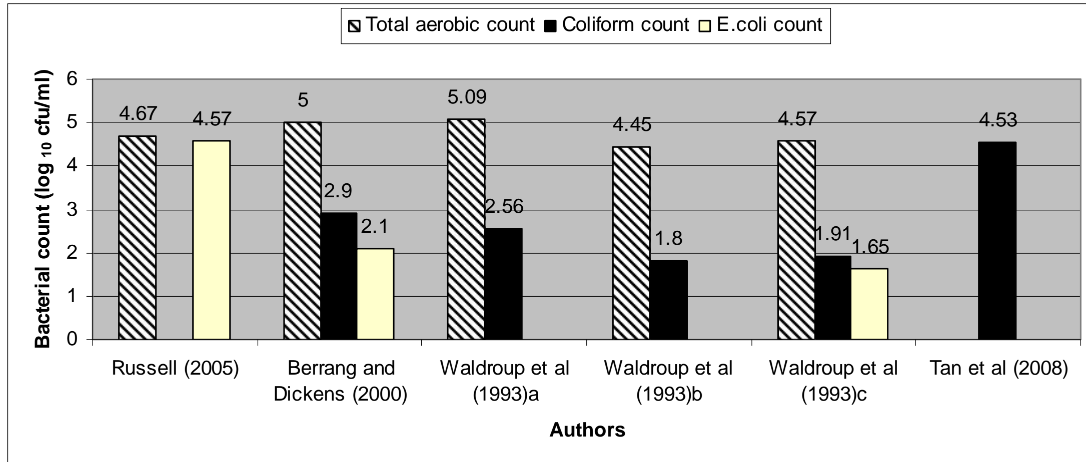

Figure 11. Post-scald carcass rinse total aerobic, coliform and *E.coli* counts shown in previous studies

The coliform levels between the post-scald and the post-pick carcass rinses decreased significantly by about 0.88 $\log_{10}$ cfu/ml. This is consistent with the study of Göksoy *et al* (2004) where the coliform counts decreased significantly by 0.23 and 0.74 $\log_{10}$ cfu/g of neck skin. This contradicts the result of the study of Berrang and Dickens (2000) where the coliform counts increased significantly by 0.5 $\log_{10}$ cfu/ml due to possible cross-contamination. Berrang and Dickens suggested two possible reasons why coliform counts would increase after the defeathering process. First, the rubber fingers might serve as a cross contaminating substrate transferring coliform bacteria to previously low-count carcasses. Secondly, the fingers might cause a jerking effect to the carcass, squeezing out fecal content and causing increased contamination. Previous post-pick carcass rinses ranged from 2.8 to 3.4 $\log_{10}$ cfu/ml (Figure 12). Unlike the post-scald data, this range is very near our post-pick coliform count of 3.64 $\log_{10}$ cfu/ml. Several new interventions like the post-pick spray or the so-called the “New York” spray have been implemented in order to counter possible cross-contamination as part of the multiple-hurdle approach. However in the study of

Stopforth *et al* (2007), the coliform count was merely reduced by 0.2 log_{10} cfu/ml which is statistically insignificant. The significant 0.88 log_{10} cfu/ml reduction after defeathering in this study can be attributed to the continuous in-process and post-pick washer employed by the plant under study.

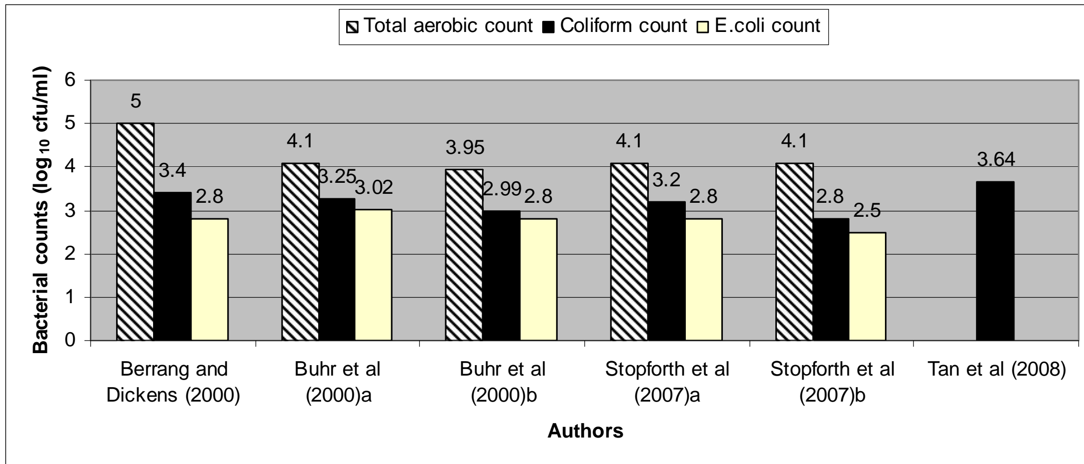

Figure 12. Post-pick carcass rinse total aerobic, coliform and *E.coli* counts shown in previous studies

The coliform levels between the rehang and the post-evisceration carcass rinses increased significantly by about 0.77 log_{10} cfu/ml. This is consistent with the study of Göksoy *et al* (2004) where the coliform counts increased, but not significantly, by 0.07 and 0.15 log_{10} cfu/g of neck skin. The evisceration step poses a huge opportunity for cross-contamination from the intestinal content (a natural reservoir for pathogenic microorganisms) to spill to the carcasses. New technology like the Nu-Tech® system that totally separates the viscera with the carcass (Russell and Walker, 1997) and added intervention like post-evisceration wash (Stopforth *et al*, 2007) may help lessen and counter the possible cross-contamination during evisceration. Automated rehang and the Nu-Tech® system were employed in the

current study. Previous post-evisceration carcass rinses ranged from 2.71 to 3.27 log_{10} cfu/ml (Figure 13). This range is slightly lower than our post-evisceration coliform counts of 3.71 log_{10} cfu/ml.

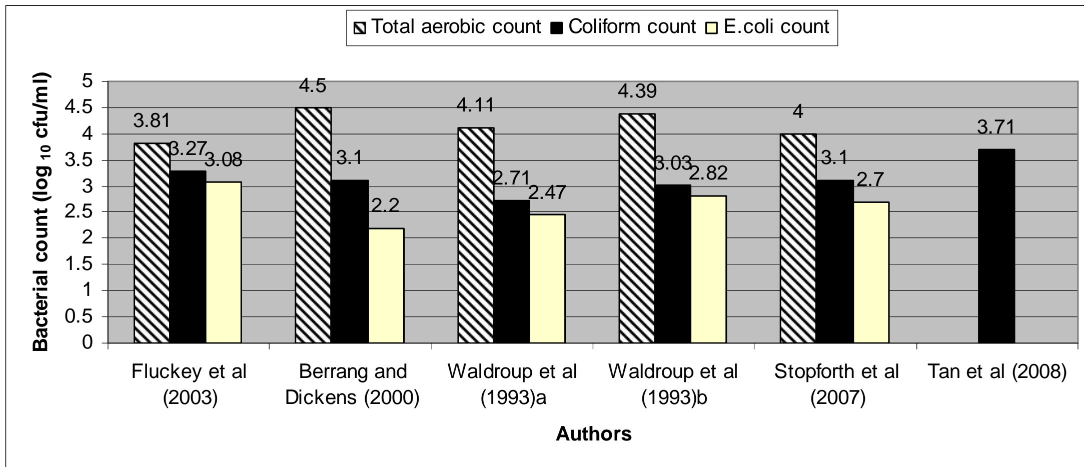

Figure 13. Post-evisceration carcass rinse total aerobic, coliform, and *E.coli* counts shown in previous studies

The coliform levels between the post-evisceration and the post-IOBW carcass rinses decreased, but not significantly, by about 0.64 log_{10} cfu/ml. This is consistent with the study of Northcutt *et al* (2003b) and Jimenez *et al* (2003) where the coliform count reductions were not significant and absent, respectively. However in the study of study of Berrang and Dickens (2000) and Stopforth *et al* (2007), the coliform counts decreased significantly. Among the factors affecting the efficiency of washers highlighted by Keener *et al* (2004) are number and types of washers, water temperature and pressure, nozzle type and arrangement, flow rate, line speed and the sanitizing agent used. In this study, the IOBW was employed only for 15 seconds. A longer washer-carcass contact time might produce a significant decrease in coliform counts. Previous post-IOBW carcass rinses ranged from 2.2 to 3.1 log_{10} cfu/ml

(Figure 14). This range is about the same as our post-IOBW coliform counts of 3.07 $\log_{10}$ cfu/ml.

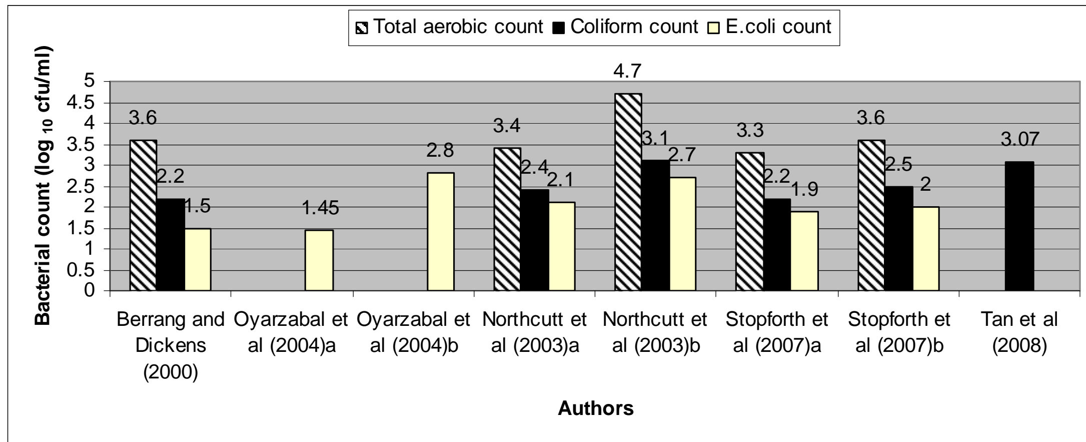

Figure 14. Post-IOBW carcass rinse total aerobic, coliform, and *E.coli* counts shown in previous studies

The coliform levels between the pre-SANOVA™ and the post-SANOVA™carcass rinse decreased significantly by about 1.42 $\log_{10}$ cfu/ml. This is consistent with several previous studies validating the tremendous effect of acidified sodium chlorite in reducing bacterial load in broiler carcasses (Kemp *et al,* 2000; Kemp *et al,* 2001; Oyarzabal *et al,* 2004; Stopforth *et al,* 2007). Using the worst case scenario (all carcasses had fecal contamination), only two out of the 1,127 carcasses tested failed to meet the USDA standard (Kemp *et al*, 2001). Kemp *et al* (2001) showed an average of 2.28 $\log_{10}$ cfu/ml decrease in *E. coli* counts.

The coliform levels between the post- SANOVA™ and the post-chill carcass rinses decreased significantly by about 0.97 $\log_{10}$ cfu/ml. However, in the continuous online processing study by Kemp *et al* (2001), the *E. coli* count increased significantly by 0.25 $\log_{10}$ cfu/ml after chilling following the ASC (acidified sodium chlorite/SANOVA™) treatment. They attributed this increase to the inability of the ASC antimicrobial to penetrate areas below the carcass surface, allowing the residual

bacterial population to survive and come out by the tumbling action during chilling, subsequently leading to a slight increase in counts. In the current study, the carcass was allowed to stay in the chiller for 90 minutes at 34^0F with 20-50 ppm chlorine. This prolonged contact time between chlorine treated chiller water with the carcass may explain the significant reduction in coliform counts. Previous post-chill carcass rinses ranged from 0.8 to 2.6 $\log_{10}$ cfu/ml (Figure 15). Our post-chill coliform counts, 0.38 $\log_{10}$ cfu/ml, were lower than this range.

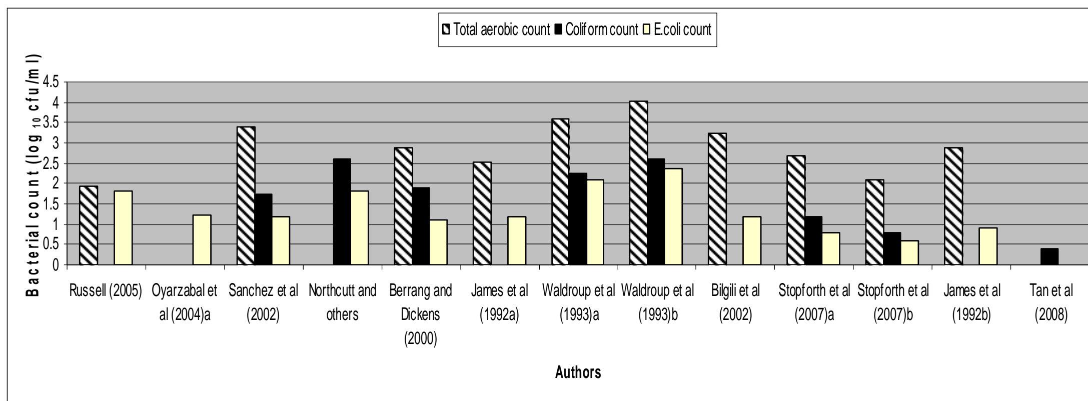

Figure 15. Post-chill carcass rinse total aerobic, coliform and *E.coli* counts shown in previous studies

Salmonella counts were only present up until the post-pick stage. All three sampling points only showed minimal *Salmonella* counts, not even reaching one $\log_{10}$ cfu/ml. Both the increase between the receiving and the post-scald and the reduction between the post-scald and the post-pick in *Salmonella* counts were statistically insignificant. These small counts can be attributed to the low sensitivity of the technique used. According to Brichta-Harhay *et al* (2007), the detection limit of the spiral plate count method in XLT4 using 50 µl in quadruplicates is only about five cfu/ml or 0.70 $\log_{10}$ cfu/ml. In this study, direct selective agar plating in XLT4 was done using 100 µl in duplicates. This detection limit is evident in the current study as

the highest average *Salmonella spp.* count was only 0.72 $\log_{10}$ cfu/ml. Direct selective enrichment, in essence, has low sensitivity because of the lack of pre-enrichment. In a comparative study between direct selective agar plating and pre-enrichment method for isolation of *Salmonella* in eggs, the pre-enrichment technique consistently produced higher isolation rates than the direct selective agar plating technique (Valentín-Bon *et al,* 2003). The sampling technique (whole-carcass enrichment) by Simmons *et* al (2003) who incubated buffered peptone water-soaked carcass for 24 hours at 37^0C before doing selective enrichment for incidence determination could be applied to enumeration studies to yield better results. However, adding an enrichment step poses a dilemma in enumeration studies because this could artificially inflate the actual counts by allowing injured *Salmonella* to recover and multiply. Other alternative methods in enumeration that can be used are real-time polymerase chain reaction (RT-PCR) and most probable number (MPN) technique. However, RT-PCR also presents some problems like low sensitivity and overestimation (Seo *et al,* 2006). MPN, likewise, has some disadvantages because it requires too much time, labor, and tubes (Seo *et al,* 2006). Most other *Salmonella* studies (Appendix J) in the processing plant entail the use of incidence/isolation rate rather than enumeration because of the difficulties cited. The American Society of Microbiology (ASM) has submitted a comment to FSIS suggesting that enumeration rather than mere isolation be used in evaluating pathogen reduction efficacy (Berkelman and Doyle, 2006). ASM contends that there is no discrimination in efficacy of pathogen reduction with just positive rates and without enumeration and that it is difficult to assess the efficacy of pathogen reduction measures without a quantitative measurement of the organism. Because it

only takes one *Salmonella* cell to produce a positive result, one carcass with ten (one $\log_{10}$ cfu/ml) *Salmonella* count and another with ten million (seven $\log_{10}$ cfu/ml) *Salmonella* count would both produce the same weighted percentage positive. An enumeration study may help identify or re-evaluate "critical control points" that could lead to new or improved interventions or methods of pathogen reduction as well as establish more accurate food safety standards. The ongoing new baseline study by the FSIS is addressing this by adding *Salmonella* enumeration (FSIS, 2007a). However, they will still be using the laborious and tedious MPN method. This poses a problem in instituting MPN enumeration of *Salmonella spp.* for HACCP systems in processing plants since chicken carcasses may well have been consumed already before the MPN results are completed and reported. Until a fast and accurate enumeration method for *Salmonella spp.* in carcass rinses can be employed, evaluation of *Salmonella* in process control would likely be limited to isolation rates.

Campylobacter spp. counts were shown to have decreased successively except after the chiller stage (Appendix K, Appendix L, and Figure 16). The *Campylobacter spp.* levels between the receiving and the post-scald carcass rinse decreased significantly by about 1.83 $\log_{10}$ cfu/ml. This is consistent with the study of Berrang and Dickens (2000) where the *Campylobacter spp.* counts decreased by 2.9 $\log_{10}$ cfu/ml. This is also consistent with the study of Izat *et al* (1988) where *Campylobacter spp.* counts decreased by about 1.84 to 2.48 $\log_{10}$ cfu/1000 cm^2 in three plants tested.

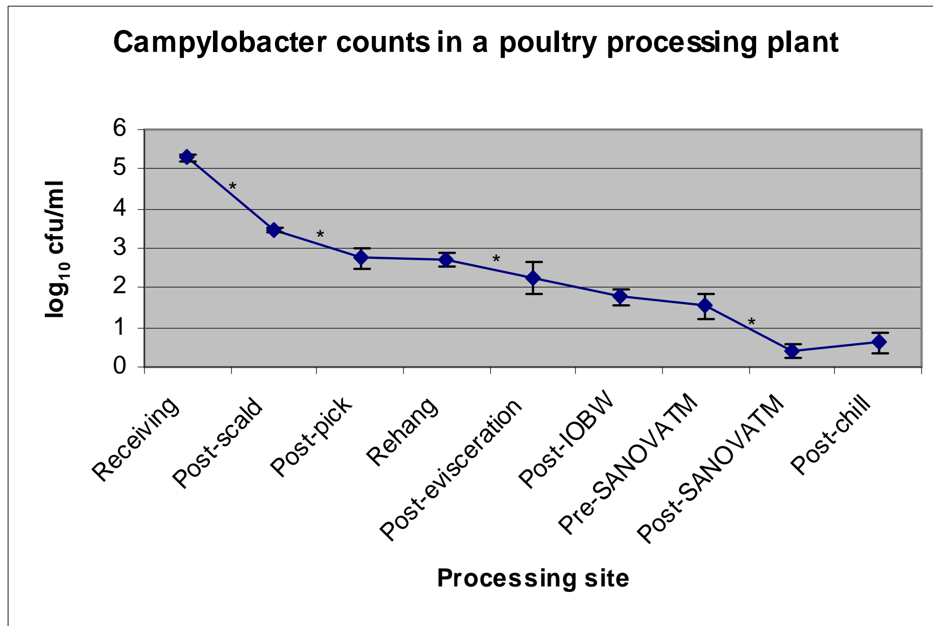

Figure 16. Line chart of average *Campylobacter spp.* counts of carcass rinses taken from different stages of a poultry processing plant
* indicates that the reduction is statistically significant

The *Campylobacter spp.* levels between the post-scald and the post-pick carcass rinse decreased significantly by about 0.71 $\log_{10}$ cfu/ml. This does not follow the result of the study of Berrang and Dickens (2000) and Izat e*t al* (1988) where the *Campylobacter spp.* counts even increased. In Berrang and Dickens (2000), the counts increased significantly by 1.9 $\log_{10}$ cfu/ml. In Izat *et al* (1988), the counts increased significantly in all three plants.

The *Campylobacter spp.* levels between the rehang and the post-evisceration carcass rinses decreased significantly by about 0.49 $\log_{10}$ cfu/ml. This is consistent with the study of Berrang and Dickens (2000) where the *Campylobacter spp.* counts decreased, but not significantly, by 0.30 $\log_{10}$ cfu/ml. Izat *et al* (1988), on the other hand, showed varying results where one plant had a significant increase, and the second and third plants had an insignificant decrease.

Just like the coliform counts, the *Campylobacter spp.* levels between the post-evisceration and the post-IOBW carcass rinses decreased, but not significantly, by about 0.49 $\log_{10}$ cfu/ml. This is consistent with the study of Berrang and Dickens (2000), Izat *et al* (1988), and Smith *et al* (2005) where there were variable *Campylobacter* count reductions. Berrang and Dickens (2000) reported significant reduction. Izat *et al* (1988) reported two plants having a significant reduction and one plant having an insignificant reduction in Campylobacter counts. Smith *et al* (2005) reported a reduction of about 1.8 $\log_{10}$ cfu/ml.

The *Campylobacter spp.* levels between the pre-SANOVA™ and the post-SANOVA™ carcass rinses decreased significantly by about 1.12 $\log_{10}$ cfu/ml. This is consistent with several previous studies validating the tremendous effect of acidified sodium chlorite in reducing bacterial load in broiler carcasses (Kemp *et al,* 2001; Oyarzabal *et al,* 2004; Bashor *et al*, 2004). Kemp *et al* (2001) showed an average of 2.56 $\log_{10}$ cfu/ml decrease in *Campylobacter spp.* counts. Bashor *et al* (2004), on the other hand, showed a significant reduction of 1.26 $\log_{10}$ cfu/ml.

The *Campylobacter spp.* levels between the post- SANOVA™ and the post-chill carcass rinse increased, albeit not significantly, by about 0.20 $\log_{10}$ cfu/ml. However, in the continuous online processing study by Kemp *et al* (2001), the *Campylobacter spp.* count decreased significantly by 0.50 $\log_{10}$ cfu/ml after chilling following the ASC (acidified sodium chlorite/SANOVA™) treatment.

It should be noted that a majority of the suspect colonies yielded negative results in the biochemical test (18/25 to 25/25) (Appendix K). Several studies has reported that Campy-Cefex agar to have a high level of contamination (false positive

colonies) (Line, 2001; Line *et al*, 2001). A more specific selective agar like the Campy-Line and Campy-Line blood agar which have additional TTC (Triphenyltetrazolium chloride) for increased resolution between the colonies and the agar should have been employed instead (Line *et al*, 2001).

Although the *Salmonella* and *Campylobacter* results in this study were less than ideal, the coliform counts did produce the intended goal of evaluating the pathogen reduction efficacy of the different stages of the processing line. Scalding, although expected to be a possible source of cross contamination, did show significantly reduced coliform counts. It is unclear whether the same level of reduction would be seen if the pre-scald point used is post-bleed rather than receiving. The said processing plant does not employ a post-scald rinse but does add chlorine in the scalding water. Picking, just like scalding, is another major concern for cross contamination. Again, picking did show a significant reduction in coliform counts. The said processing plant does employ continuous wash during defeathering and uses a post-picking washer. Added attention must be given in the evisceration stage because there was a statistically significant increase in coliform counts. Rehang in this plant was reported to be automated. The Nu-Tech® evisceration system is employed. The IOBW final wash did result in an effective reduction in this plant, albeit not significant. The online reprocessing antimicrobial step resulted in the largest reduction of almost 1.5 $\log_{10}$ cfu/ml. The chiller tank, just like the scald tank, also presents the risk of cross contamination. This particular plant showed that even the chiller significantly reduced coliform counts by almost one $\log_{10}$ cfu/ml. The final average post-chill coliform count was 0.38 $\log_{10}$ cfu/ml. This is much lower than the

FSIS latest baseline generic *E. coli* limit for acceptable results for carcass rinse of young chickens (35 cfu/ ml or about 1.54 log_{10} cfu/ml) (FSIS, 2005) and even much lower than the HACCP established minimum performance benchmark of 100 cfu/ml or two log_{10} cfu/ml for lower limit (FSIS, 1996b). The final average post-chill *Campylobacter spp.* count was 0.61 log_{10} cfu/ml. This is almost equivalent to the baseline data by Berrang *et al* (2007) for post-chill carcass rinse (0.43 log_{10} cfu/ml). Based on the critical control point decision tree (FSIS, 1996b) and considering that only the post-chill carcass rinse has a nationwide official baseline acceptable level, we can only conclude that the chiller is indeed a critical control point. Furthermore, this baseline study may serve as a control for future studies by this plant whenever process control modification is implemented just like what was done by James *et al* (1992a) and James *et al* (1992b).

Chapter 6: Comparison of Two Online Reprocessing (OLR) Antimicrobials

6.1 Review of Literature

In the past, the reprocessing rate in poultry processing plants has averaged between 2 to 5 % (about 20,000 to 50,000 birds per week) (Fletcher *et al*, 1997). Current authors estimate the reprocessing rate at 0.5 to 1 % (Russell, 2007a). Prior to 1989, carcasses that have accidentally been contaminated by feces during the evisceration process were subjected to manual trimming in an off-line site (Russell, 2007a). This process, according to Russell (2007a), poses a lot of problems by virtue of being labor intensive and triggers various probems associated with offline processing such as: labor cost, labor issues (absenteeism), work-related injuries, and the possibility of cross contamination (due to manually transporting the contaminated carcass to an offline site and rehanging to the chiller). In addition, if the fecal contamination is in the inside cavity, there would be no way to trim the carcass and hence, it will be disposed of (Russell, 2007a). According to the rule published by the USDA Food Safety and Quality Services in the Federal Register in 1978, procedures such as trimming, vacuuming, washing or any combinations of such will be permitted as reprocessing tools. In addition, if inner surfaces are reprocessed other than by trimming, all the surfaces of the carcass must be treated with 20 ppm chlorine solution (Rasekh *et al,* 2005). Initially, 20 ppm chlorine was instituted as a reprocessing tool in an offline reprocessing station in accordance with the 1978 rule. The USDA-FSIS allows up to 50 ppm chlorine in water for carcass washer

application and chiller water (Russell and Keener, 2007). According to a study by Waldroup *et al* (1993a), 4 out of the 5 plants studied had carcasses with statistically significant lower aerobic plate, coliform, and *E.coli* counts when reprocessed with 20 ppm chlorine compared to the inspection passed carcasses. Supporting the justification for the use of online reprocessing, Fletcher *et al* (1997) undertook a unique study that is usually quoted in current literature. Visually contaminated carcasses after evisceration deemed by USDA line inspectors to be suitable for reprocessing offline, were used as a criterion as "test" samples and were tested against the control (those that passed the visual inspection after the chlorinated IOBW). Fletcher *et al* (1997) showed that 67 % of carcasses that were being removed by line inspectors, upon closer inspection, actually had no visual contamination and could have been processed online. Adding those that have only a visual contamination score of 1 (only 1 speck), which were found to be almost all effectively eliminated by the IOBW in the control, a total of 81 % of carcasses pulled for reprocessing would have been cleaned sufficiently later on by the IOBW if left in the line. Furthermore, Fletcher *et al* (1997) showed that carcass processing using online reprocessing had no difference in aerobic plate, coliform, and *Campylobacter* counts compared to those that were manually reprocessed, alleviating initial consumer concerns at that time that online reprocessing may not be as effective as manual reprocessing. The development of continuous online reprocessing systems soon followed with the use various new antimicrobial solutions developed other than chlorine. Among these antimicrobial systems are: trisodium phosphate, acidified sodium chlorite (SANOVA^{TM}), peracetic acid and combinations of peracetic acid and

octanoic acid (FMC 323® and Inspexx 100®), chlorine dioxide, acidified chlorine (TomCo®), cetypyridinium chloride (Cecure®), mixture of acids (SteriFx® FreshFx®), and acidified calcium sulfate (Safe$_2$O®) (Russell, 2007a). Some other antimicrobial products tested such as lactic acid and sodium bisulfate produced slight discoloration of carcasses (Yang *et al*, 1998).

According to the survey undertaken by Russell (2007b) from 94 plants in the US, acidified sodium chlorite (SANOVA™) by far is the most commonly used by companies (33 percent). This is followed by trisodium phosphate (Rhodia®) (24%), chlorine dioxide (15%), hypochlorous acid (9%), organic acids (6%), peracetic acid (Perasafe®) (5%) and cetylpyridinium chloride and others making up the rest. However, according to Rasekh *et al* (2005), 80 plants in the U.S. are currently using TSP (trisodium phosphate) and 38 are using acidified sodium chloride.

Acidified sodium chlorite/ASC (Sanova Food Quality System/SANOVA™) is a mixture of sodium chlorite ($NaClO_2$) and a generally recognized as safe (GRAS) organic acid which is usually citric acid. There have been several publications assessing the efficacy of ASC in decreasing bacterial counts in poultry carcasses when used as an antimicrobial agent for online reprocessing. Oxychlorous intermediate is formed instantaneously when sodium chlorite and organic acid are combined and come into contact with organic matter (Kemp *et al*, 2000). This level increases when the solution decreases from pH 4 (Keener *et al*, 2004). The approved dose for ASC is 500 to 1200 ppm to achieve a working pH of 2.3 to 2.9 in automated reprocessing (Bennett, 2006). The mechanisms of action of ASC are oxidizing the cell wall, attacking the sulfide and non-sulfide linkages in proteins, and non-

specifically attacking the amino acid of the cell membrane (Keener *et al,* 2004). Kemp *et al* (2000) cited an unpublished report by Kemp (2000) that none of 10 microbes tested developed resistance after more than 100 divisions when placed in sub-inhibitory dose of ASC. Kemp *et al* (2000) made a series of experiments to evaluate different treatment parameters for ASC use. They found that ASC worked better when carcasses are pre-washed than not, given at higher concentration (1200ppm) than lower concentration (500 and 850 ppm) and applied by dipping than spraying. There was no difference found when either citric acid or phosphoric acid was used as the acidifier. The reason why citric acid is commonly used is because of the apparent additional chelating activity it confers to the antimicrobial and the concerns about disposing phosphate containing waste (Kemp *et al*, 2000) Kemp *et al* (2000) also noted mild but transient skin whitening in the carcass at 1200 ppm that was lost when subjected to the chiller. In a different study, Kemp *et al* (2001) showed that combined reduction effects of final washer and ASC spray (continuous online processing/COP) is much better than subjecting carcasses to offline reprocessing in terms of *E. coli* and *Campylobacter* counts as well as *Salmonella* and *Campylobacter* incidence rate. Considering all the samples used were initially visibly contaminated, the COP was able to reduce *E.coli* by almost 2 logs and *Campylobacter* by about 1.5 logs more than offline reprocessing (Kemp *et al*, 2001). On the other hand, Bashor *et al* (2004) reported that combined IOBW and ASC rinses decreased *Campylobacter* by 1.52 log cfu/ml. Kemp and Schneider (2000) showed that *E. coli* counts with increasing ASC concentration in buffered peptone water (BPW) remained constant, validating that BPW alone can neutralize the effect of ASC for enumeration purposes.

Perasafe® is composed of 15% peracetic acid and 10% hydrogen peroxide (anonymous). The oxidizing activity of hydrogen peroxide causes disruption of the cell membrane and protein synthesis through reaction with sulfhydryl, sulfide, amino acid containing disulfide and nucleotide. In addition, the antimicrobial actions of peracetic acid are acidifying the carcass surfaces and allowing the penetration of acids into bacteria (Oyarzabal, 2005). Currently, there is no published literature on assessing the use of Perasafe® as an online reprocessing antimicrobial. Since peracetic acid may react with blood vessels, slight gray discoloration of the carcass may occur, especially in highly vascular areas such as the neck (Russell, 2007b). In an experiment by Dickens and Whittemore (1997) determining the separate effects of acetic acid and hydrogen peroxide in microbial reduction, 1 % acetic acid was found to have 0.6 log cfu/ml more reduction in total aerobic counts than controls while 3 different concentrations of hydrogen peroxide were found to have no increase in reduction of total aerobic counts compared to controls. However, it should be noted that the contact time for both antimicrobials is only 30 seconds. In a study by Chantarapanont *et al* (2004), the GFP-*Campylobacter jejuni* count was decreased by 1.05 log cfu/ml when 100 ppm peracetic acid was applied for 15 minutes as a dip in an attachment assay.

6.2 Materials and Methods

6.2.1 Sample Size and Collection

Ten carcass samples were taken daily for 8 days from both line 1 and line 2 at three processing points namely: pre-OLR (online reprocessing), post-OLR, and post-

chill. Samples came from different flocks on different days of collection and were collected simultaneously from line 1 and line 2.. Each whole carcass was placed in a bag of 100 ml buffered peptone (Becton, Dickenson and Company, Sparks, MD) and shaken manually in the so called "shake and bake" method for one minute. Fifty ml of the carcass rinse from each bag were then placed in a cooler with ice packs and sent to the Lasher Poultry Diagnostic Laboratory in Georgetown, Delaware.

6.2.2 Bacterial Enumeration

Upon receiving the samples, serial dilutions up to 1:100 were performed. One ml of each sample dilution was inoculated into 3M® Petrifilm® Total Aerobic Count Plate and Coliform Count Plate (3M Microbiology Products, St. Paul, MN) for total aerobic and coliform counts, respectively. The cover film was then placed and the inoculum was spread evenly on the Petrifilm® using a weight spreader. The films were incubated at 37 ^{0}C for 24 hrs before counting (Russell, 2000).

6.2.3 Statistical Analysis

All bacterial counts of 0 were replaced with 1 to allow log transformation. All statistical measures were done in $\log_{10}$ cfu/ml unit. Using Statistical Analysis System/SAS (SAS Institute Inc., Cary, NC)'s PROC GLM (General Linear Model), the absolute difference between bacterial counts for each pair of consecutive processing points from one OLR antimicrobial agent were tested statistically against that of the other OLR antimicrobial agent. This was calculated using with a contrast formula: +1 -1 -1 +1. A GLM table between the Line, Trial (Day) and their interaction was constructed using pre-OLR data.

6.3 Results and Discussion

Both the two online reprocessing antimicrobials produced less than 1 $\log_{10}$ cfu/ml reduction for TAC after OLR treatment (Appendix M, Figure 17, and Figure 18). However, TAC counts were reduced by almost 2 $\log_{10}$ cfu/ml after chilling. This observation seems to suggest a synergistic effect between the OLR antimicrobial and the chlorine at the chill tank. This possible synergism was suggested by Oyarzabal *et al* (2004). However, their experiment was in reverse of the current experiment where acidified sodium chlorite dip was applied after chilling. These researchers suggested that the chlorine and cold temperature during the chilling process had weakened the bacterial cell wall, allowing acidified sodium chlorite to produce tremendous reduction. It is unclear whether OLR or chilling, if applied first, would have a better effect. A future comparative study is suggested. Coliform counts, likewise, were reduced only by about 1 $\log_{10}$ cfu/ml post-OLR but about 2 $\log_{10}$ cfu/ml post-chill for both antimicrobials (Appendix M, Figure 19, and Figure 20).

All differences of reduction in TAC and coliform counts between the two antimicrobials were found to be statistically insignificant both in individual lines and lines combined (Appendix N). The difference in counts between pre-OLR and post-OLR, between the pre-OLR and post-chill, and between the post-OLR and post-chill would represent the effect of the OLR antimicrobial, combined effects of OLR antimicrobial and chlorine in the chill tank, and the chlorine in the chill tank, respectively. The majority of these combinations suggest that Perasafe® has a slight edge in bacterial reduction than SANOVA™, although this difference is statistically insignificant. The main purpose of this study was to determine whether using a new

OLR antimicrobial (Perasafe®) would be more cost-effective than the currently used antimicrobial (SANOVA™) Our study showed that there was no statistically significant difference in performance between the two antimicrobials.

As a side study, the two lines were compared at the pre-OLR stage to determine whether there was a difference in performance between the two lines with regards to the evisceration and IOBW. There was a statistical difference between lines in terms of coliform counts but not TAC (Appendix O). Line 1 showed lower coliform counts than line 2 (Appendix P). In fact, it is obvious that Line 2 had higher post-OLR coliform counts than Line 1 (Figure 19 and Figure 20). There were statistically significant differences in both TAC and coliform counts in regards to day of collection. Both TAC and coliform counts were lowest at day 2 of collection. Day 6 and day 1 resulted in the highest TAC and coliform counts, respectively. There was a significant interaction between day of collection and line for coliform counts but not for TAC counts. Line 1 at day 2 of collection showed the lowest coliform count while line 2 of day 6 showed the highest coliform count.

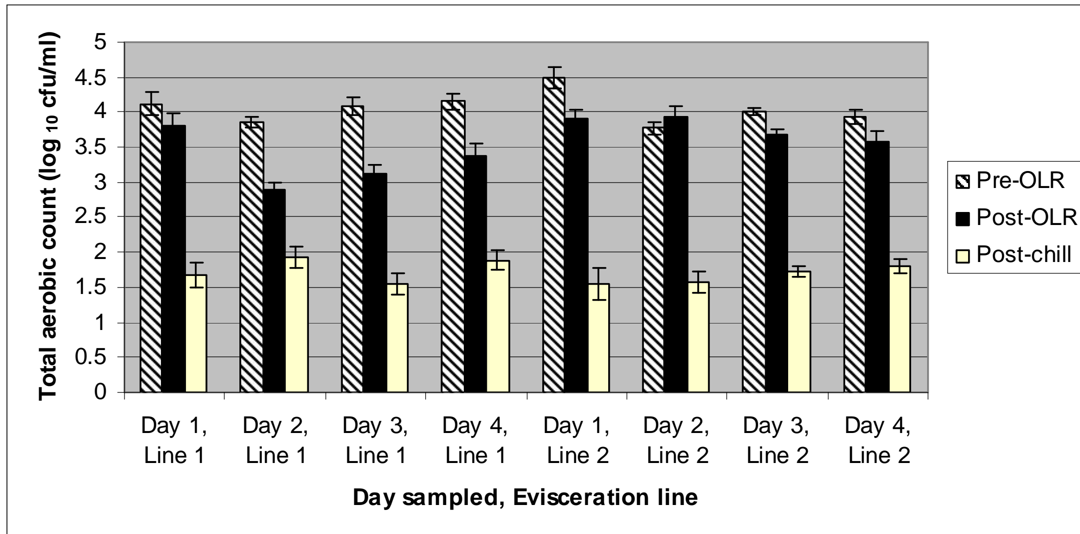

Figure 17. Average total aerobic counts (TAC) of carcass rinses taken from pre-OLR, post-OLR and post-chill stages of the processing plant using SANOVA™ antimicrobial
OLR=online reprocessing

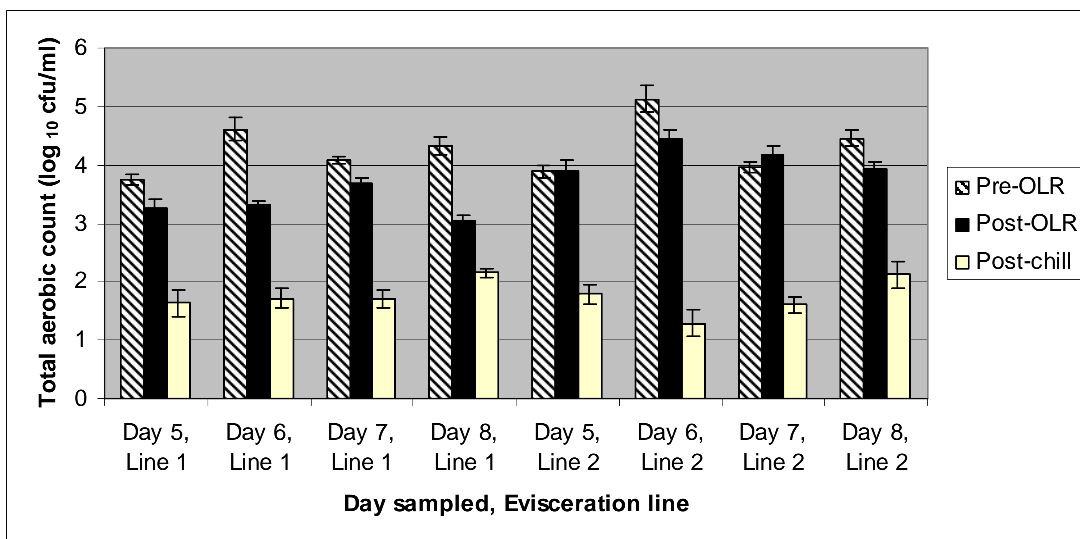

Figure 18. Average total aerobic counts (TAC) of carcass rinses taken from pre-OLR, post-OLR and post-chill stages of the processing plant using Perasafe® antimicrobial
OLR=online reprocessing

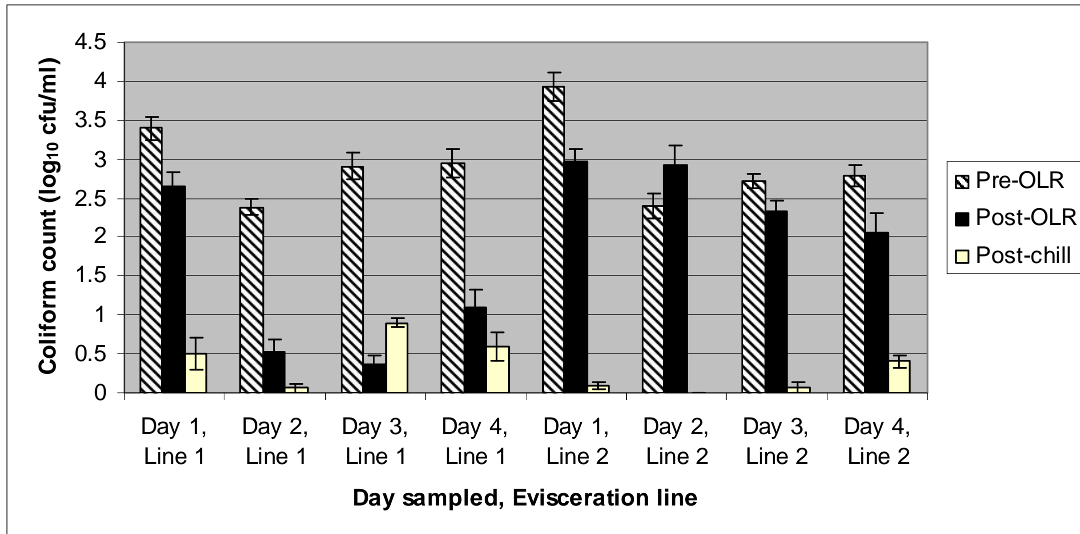

Figure 19. Average coliform counts of carcass rinses taken from pre-OLR, post-OLR and post-chill stages of the processing plant using SANOVA™ antimicrobial OLR=online reprocessing

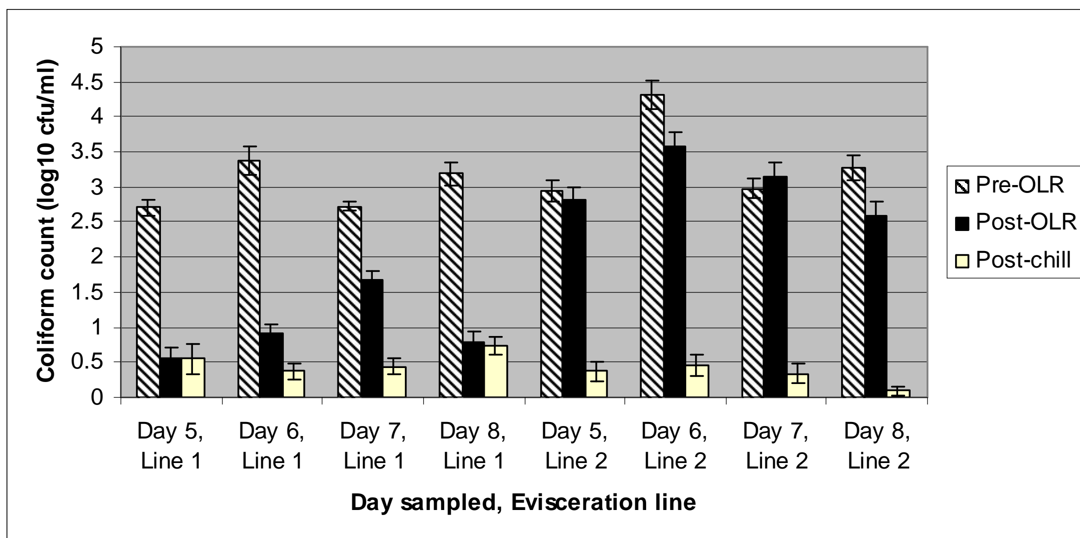

Figure 20. Average coliform counts of carcass rinses taken from pre-OLR, post-OLR and post-chill stages of the processing plant using Perasafe® antimicrobial OLR=online reprocessing

Chapter 7: Summary and Conclusion

Although the *Clostridium perfringens* counts in gangrenous dermatitis affected farms were statistically significantly higher than those in non-affected (control) farms, the effective difference was only about 0.35 $\log_{10}$ cfu/ml or equivalent to about 183 cfu/ml. The results of the comparative litter survey study suggest that gangrenous dermatitis-affected farms may not signify an effective increase in the level of the food-poisoning agent *Clostridium perfringens* type A. Considering that previous studies have indicated that only about 5% of the global *Cl. perfringens* isolates are positive to *cpe gene* (McClane, 2001), future survey studies should include typing of isolates with this gene. Factoring the block and location differences, only the soil pads during harvest (week 7) at the west house had a statistically significant difference in *Clostridium perfringens* counts among treatments with the PLT® sub-plot, resulting in lower counts than the controls (no treatment) and the salt sub-plots. However, the effective difference in *Cl. perfringens* counts between PLT® and control sub-plots was only about 0.12 $\log_{10}$ cfu/ml or about 1.32 cfu/ml. Since the current grow-out practice is to re-use the litter for several grow-out cycles, it is recommended that litter amendments also be applied to the soil pad as it might serve as a reservoir of foodborne pathogens, particularly, *Clostridium perfringens.* The litter amendment study showed that *Clostridium perfringens* counts in litter from both houses were declining over time. In the windrow-composting studies, two houses each showed a significant decrease in total aerobic and coliform counts as

well as in *Salmonella spp.* incidence. In addition, all four houses tested showed numerical decreases in *Clostridium perfringens* counts. Although the *Salmonella* and *Campylobacter* results of the processing line study were less than ideal, the coliform counts did produce the intended goal of evaluating the pathogen reduction efficacy of the different stages of the processing line. All processing steps except the evisceration and IOBW resulted in a statistically significant reduction in coliform counts. All processing steps except the IOBW and chiller resulted in a statistically significant reduction in *Campylobacter spp.* counts. Since studies have reported that Campy-Cefex agar has a high level of contamination (false positive colonies) (Line, 2001; Line *et al*, 2001), a more specific selective agar like the Campy-Line and Campy-Line blood agar is recommended for future processing plant baseline studies. In the comparative OLR antimicrobial study, both TAC and coliform counts were reduced only by about 1 $\log_{10}$ cfu/ml after the OLR but were reduced by 2 $\log_{10}$ cfu/ml after the succeeding chiller, suggesting a synergistic effect between the OLR antimicrobial and the chiller chlorine. It is suggested that a comparative study be done to compare the effect when OLR is used before and after the chiller. The differences in reduction between the two commercial antimicrobials were found to be statistically insignificant. Line two of the comparative antimicrobial study showed a statistically significant higher coliform count than line one suggesting that line 2 was more prone to coliform contamination during the evisceration and IOBW stages of processing. Similar in-house studies may be adopted by poultry integrators in their processing plants to identify problem areas promptly in order to initiate timely corrective and preventive actions. Finally, intervention strategies during farm production and

processing were found to be able to help reduce the level of common foodborne pathogens.

Appendices

Appendix A. Average total aerobic, coliform and *Clostridium perfringens* counts in litter of gangrenous dermatitis-affected and control farms

	Total Aerobic Counts (log_{10} cfu/ml±SE)	Coliform Counts (log_{10} cfu/ml±SE)	*Clostridium perfringens* Counts (log_{10} cfu/ml±SE)
Gangrenous Dermatitis-Affected Farms			
Farm A (n=10)	6.57±0.14	0.10±0.10	3.00±0.14
Farm B (n=10)	5.81±0.17	1.18±0.50	2.15±0.10
Farm C (n=10)	6.49±0.22	1.23±0.38	2.80±0.13
Farm D (n=10)	7.41±0.17	1.12±0.34	2.15±0.17
Farm E (n=10)	7.30±0.21	1.83±0.37	2.07±0.18
Farm F (n=10)	7.83±0.10	2.29±0.33	2.27±0.08
Farm G (n=10)	5.81±0.16	1.06±0.45	3.04±0.16
Farm H (n=9)	7.58±0.20	3.88±0.31	2.67±0.13
Total (n=79)	6.84±0.10	1.56±0.17	2.52±0.06
Control Farms			
Farm I (n=10)	6.41±0.15	0.27±0.27	2.23±0.26
Farm J (n=10)	6.37±0.13	1.44±0.58	2.44±0.13
Farm K (n=10)	7.12±0.19	2.53±0.35	1.83±0.31
Total (n=30)	6.64±0.11	1.41±0.29	2.17±0.15

Appendix B. ANOVA table for total aerobic, coliform and *Clostridium perfringens* counts in litter of gangrenous dermatitis-affected and control farms

	Mean Square	F-value	P-value	Interpretation
Total Aerobic Counts				
Farm Status	0.99179239	3.45	0.0661	Not significant
Farm (Farm Status)[a]	5.21042813	18.15	<0.0001	Significant
Coliform Counts				
Farm Status	0.6566057	0.46	0.5014	Not significant
Farm (Farm Status)[a]	12.0402945	8.35	<0.0001	Significant
Cl. perfringens Counts				
Farm Status	2.700011400	8.75	0.0039	Significant
Farm (Farm Status)[a]	1.148555864	4.81	<0.0001	Significant

[a]Farm (Farm Status) row is the analysis of variance between all farms nested in their respective farm status category

$\alpha = 0.05$

Appendix C. Average *Clostridium perfringens* counts in soil and litter using PLT® (Sodium Bisulfate), salt and no treatments

	Control (log_{10} cfu/ml±SE) East: n=15 West: n=15	PLT® (log_{10} cfu/ml±SE) East: n=15 West: n=15	Salt (log_{10} cfu/ml±SE) East: n=15 West: n=15
Litter, Week 3	East: 2.23±0.12 West: 2.22±0.14	East: 2.28±0.13 West: 2.03±0.10	East: 2.36±0.11 West: 2.05±0.15
Litter, Week 5	East: 1.63±0.13 West: 1.78±0.21	East: 1.53±0.22 West: 1.93±0.19	East: 1.57±0.17 West: 1.79±0.20
Litter, Week 7	East: 1.06±0.18 West: 1.19±0.21	East: 1.31±0.13 West: 1.21±0.18	East: 1.19±0.21 West: 1.16±0.18
Soil, Week 7	East: 0.50±0.13 West: 0.19±0.07	East: 0.34±0.11 West: 0.80±0.05	East: 0.56±0.15 West: 0.46±0.19

Each *Cl. perfringens* count is an average of duplicates.

Appendix D. Results of t-test between two treatment means of the litter amendment study

	Treatment 1 n=15	Treatment 2 N=15	Difference (log_{10} cfu/ml)	T-value	P-value	Interpretation
Litter, Week 3, East	Control	PLT®	-0.0550	-0.31	0.7615	Not Significant
	Control	Salt	-0.1310	-0.81	0.4230	Not Significant
	PLT®	Salt	-0.0760	-0.44	0.6666	Not Significant
Litter, Week 3, West	Control	PLT®	0.1908	1.12	0.2703	Not Significant
	Control	Salt	0.1644	0.80	0.4332	Not Significant
	PLT®	Salt	-0.0260	-0.15	0.8848	Not Significant
Litter, Week 5, East	Control	PLT®	0.0999	0.39	0.6969	Not Significant
	Control	Salt	0.0563	0.26	0.7966	Not Significant
	PLT®	Salt	-0.0440	-0.16	0.8764	Not Significant
Litter, Week 5, West	Control	PLT®	-0.1530	-0.55	0.5884	Not Significant
	Control	Salt	-0.0100	-0.03	0.9733	Not Significant
	PLT®	Salt	0.1428	0.52	0.6060	Not Significant
Litter, Week 7, East	Control	PLT®	-0.2510	-1.14	0.2649	Not Significant
	Control	Salt	-0.1250	-0.45	0.6537	Not Significant
	PLT®	Salt	0.1264	0.52	0.6093	Not Significant
Litter, Week 7, West	Control	PLT®	-0.0240	-0.09	0.9324	Not Significant
	Control	Salt	0.0253	0.09	0.9271	Not Significant
	PLT®	Salt	0.0491	0.19	0.8473	Not Significant
Soil, Week 7, East	Control	PLT®	0.1573	0.90	0.3751	Not Significant
	Control	Salt	-0.0640	-0.32	0.7507	Not Significant
	PLT®	Salt	-0.2210	-1.19	0.2449	Not Significant
Soil, Week 7, West	Control	PLT®	0.1121	1.33	0.1947	Not Significant
	Control	Salt	-0.2650	-1.32	0.1969	Not Significant
	PLT®	Salt	-0.3770	-1.95	0.0610	Not Significant

$\alpha = 0.05$

Appendix E. GLM table for *Clostridium perfringens* counts in litter and soil of the litter amendment study

	Source	Mean Square	F-value	P-value	Interpretation
Soil, Baseline, East	Block	0.35102628	0.91	0.433	Not Significant
	Location	0.99216300	2.57	0.125	Not significant
Soil, Baseline, West	Block	1.05425566	3.06	0.091	Not significant
	Location	0.54600500	1.59	0.252	Not significant
Litter, Week 3, East	Block	1.78693089	12.42	<0.0001	Significant
	Location	0.36898466	2.56	0.091	Not significant
	Treatment	0.06901766	0.48	0.623	Not significant
	Location*Treatment	0.02806349	0.20	0.939	Not significant
Litter, Week 3, West	Block	1.32867156	5.98	0.005	Significant
	Location	0.20026610	0.90	0.415	Not significant
	Treatment	0.12755570	0.57	0.568	Not significant
	Location*Treatment	0.08570995	0.39	0.817	Not significant
Litter, Week 5, East	Block	3.35047128	14.49	<0.0001	Significant
	Location	2.56260871	11.08	0.0001	Significant
	Treatment	0.03865046	0.17	0.846	Not significant
	Location*Treatment	0.02377550	0.10	0.980	Not significant
Litter, Week 5, West	Block	6.40124711	21.73	<0.0001	Significant
	Location	0.46107862	1.57	0.223	Not significant
	Treatment	0.06976400	0.24	0.790	Not significant
	Location*Treatment	0.24758144	0.84	0.509	Not significant
Litter, Week 7, East	Block	3.47305835	13.40	<0.0001	Significant
	Location	1.01662431	3.92	0.029	Significant
	Treatment	0.30340765	1.17	0.322	Not significant
	Location*Treatment	0.38242228	1.47	0.231	Not significant
Litter, Week 7, West	Block	1.82546011	4.07	0.026	Significant
	Location	1.17803680	2.63	0.087	Not significant
	Treatment	0.11164873	0.25	0.781	Not significant
	Location*Treatment	0.36097762	0.80	0.530	Not significant
Soil, Week 7, East	Block	0.53332026	2.90	0.069	Not significant
	Location	0.79522848	4.32	0.021	Significant
	Treatment	0.48536326	2.63	0.086	Not significant
	Location*Treatment	0.50854518	2.76	0.043	Significant
Soil, Week 7, West	Block	0.03752777	0.19	0.831	Not significant
	Location	0.30673472	1.51	0.234	Not significant
	Treatment	0.73764568	3.64	0.036	Significant
	Location*Treatment	0.32694706	1.61	0.193	Not significant

$\alpha = 0.05$

Appendix F. Least square means of *Clostridium perfringens* counts in soil and litter based on block, location, and treatment

	Block	log_{10} cfu/ml±SE	Location	log_{10} cfu/ml±SE	Treatment	log_{10} cfu/ml±SE
Soil, Baseline, East	Door	1.25±0.28	Center	1.84±0.36		
	Fan	1.73±0.28	Side	1.05±0.25		
	Middle	1.68±0.28	Waterer	1.76±0.25		
Soil, Baseline, West	Door	0.33±0.27	Center	1.24±0.34		
	Fan	0.80±0.27	Side	0.56±0.24		
	Middle	1.25±0.27	Waterer	0.58±0.24		
Litter, Week 3, East	Door	2.60±0.10	Center	2.36±0.13	Control	2.23±0.10
	Fan	1.93±0.10	Side	2.14±0.09	PLT®	2.32±0.10
	Middle	2.38±0.10	Waterer	2.41±0.09	Salt	2.37±0.10
Litter, Week 3, West	Door	1.76±0.12	Center	2.12±0.16	Control	2.21±0.13
	Fan	2.22±0.12	Side	1.99±0.11	PLT®	2.04±0.13
	Middle	2.32±0.12	Waterer	2.20±0.11	Salt	2.05±0.13
Litter, Week 5, East	Door	1.98±0.13	Center	1.48±0.16	Control	1.62±0.13
	Fan	1.05±0.13	Side	1.23±0.11	PLT®	1.52±0.13
	Middle	1.65±0.13	Waterer	1.97±0.11	Salt	1.54±0.13
Litter, Week 5, West	Door	1.05±0.14	Center	1.60±0.18	Control	1.74±0.15
	Fan	2.26±0.14	Side	1.79±0.13	PLT®	1.88±0.15
	Middle	2.07±0.14	Waterer	1.98±0.13	Salt	1.76±0.15
Litter, Week 7, East	Door	1.59±0.13	Center	0.98±0.17	Control	1.05±0.14
	Fan	0.64±0.13	Side	1.03±0.12	PLT®	1.32±0.14
	Middle	1.23±0.13	Waterer	1.45±0.12	Salt	1.08±0.14
Litter, Week 7, West	Door	0.93±0.18	Center	1.41±0.22	Control	1.23±0.18
	Fan	1.13±0.18	Side	0.91±0.16	PLT®	1.31±0.18
	Middle	1.61±0.18	Waterer	1.35±0.16	Salt	1.13±0.18
Soil, Week 7, East	Door	0.30±0.11	Center	0.72±0.14	Control	0.52±0.12
	Fan	0.66±0.11	Side	0.25±0.10	PLT®	0.32±0.12
	Middle	0.58±0.11	Waterer	0.56±0.10	Salt	0.70±0.12
Soil, Week 7, West	Door	0.24±0.12	Center	0.43±0.15	Control	0.20±0.12
	Fan	0.33±0.12	Side	0.12±0.11	PLT®	0.08±0.12
	Middle	0.25±0.12	Waterer	0.28±0.11	Salt	0.53±0.12

Appendix G. Average total aerobic, coliform, and *Clostridium perfringens* counts and prevalence rate of *Salmonella spp.* in litter pre- and post- windrow composting

	Total Aerobic Counts (log_{10} cfu/ml±SE)	Colifrom Counts (log_{10} cfu/ml±SE)	*Clostridium perfringens* Counts (log_{10} cfu/ml±SE)	*Salmonella spp.* incidence rate [no. positive/total (%)][b]
House A				
Pre-windrow (n=10)	4.08±0.11	Undetectable	2.34±0.27	0/10 (0%)
Post-windrow (n=10)	6.37±0.14[a]	Undetectable	1.56±0.09	0/10 (0%)
House B				
Pre-windrow (n=10)	6.08±0.04[a]	Undetectable	2.36±0.01	0/10 (0%)
Post-windrow (n=10)	6.80±0.04	Undetectable	1.73±0.06	0/10 (0%)
House C				
Pre-windrow (n=10)	7.41±0.17	1.12±0.34	2.15±0.17	4/10 (40%)
Post-windrow (n=10)	6.58±0.18	Undetectable	1.95±0.27	1/10 (10%)
House D				
Pre-windrow (n=60)	6.89±0.09	22.2±7.62[c]	2.23±0.10	2/12 (17%)[d]
Post-windrow (n=12)	4.98±0.21	Undetectable	0.98±0.24	0/12 (0%)

All houses are affected by gangrenous dermatitis
[a]n=9, one sample was uncountable
[b]Suspected *Salmonella* colonies were confirmed with API20E
[c]Unit is cfu/ml because the counts were too low
[d]n=12 instead of n=60, 1 sample represents 5 pooled original samples

Appendix H. Results of t-test between two means (pre- and post-windrow) for total aerobic, coliform. and *Clostridium perfringens* counts in litter samples

	Mean Difference (log_{10} cfu/ml)	T-value	P-value	Interpretation
House A n=20				
Total Aerobic Count	-2.2890	-12.9	<0.0001	Significant
Clostridium perfringens count	0.7864	2.72	0.0196	Significant
House B n=20				
Total Aerobic Count	-0.7200	-12.7	<0.0001	Significant
Clostridium perfringens count	0.6270	7.77	<0.0001	Significant
House C n=20				
Total Aerobic Count	0.8358	3.39	0.0032	Significant
Clostridium perfringens count	0.3905	1.22	0.2394	Not significant
House D n=72				
Total Aerobic Count	1.9078	8.92	<0.0001	Significant
Coliform Count	22.2[a]	2.91	0.0050	Significant
Clostridium perfringens count	1.2439	5.17	<0.0001	Significant

[a]Unit is cfu/ml because the counts were too low

$\alpha = 0.05$

Appendix I. Aerobic, coliform, enterbactericeae, and *E. coli* populations measured in poultry processing by various researchers

Stages of Processing	Total aerobic population (log_{10} cfu/ml)	Coliform and Enterobacteriaceae population (log_{10} cfu/ml) C=coliform E=enterobacteriaceae	*E. coli* population (log_{10} cfu/ml)
Farm	1-day before processing: litter drag swab, 6.95[d]	1-day before processing: litter drag swab, (C) 4.24[d]	1-day before processing: litter drag swab, 3.88[d]
Transportation/initial level entering plant	Post-kill: carcass, 4.72[a] Post-kill: neck skin, 7.4 & 7.56[c] Post-kill: carcass, 6.8[g] Post-stun: carcass, 7.17 & 6.96[h] Post-kill: breast skin, 6.52[j] Post-kill: neck skin, 5.2[m] Post-kill: neck skin, 6.4, 6, 5.2, 6.3 & 6.4[p]	Post-kill: neck skin, (C) 5.35 & 5.67[c] Post-kill: neck skin, (E) 5.36 & 5.75[c] Post-kill: carcass, (C) 5[g] Post-stun: carcass, (C) 6.2 & 5.63[h] Post-kill: breast skin, (E) 4.76[j] Post-kill: neck skin, (E) 4.8[m] Post-kill: neck skin, (C) 4.6[m] Post-kill: neck skin, (C) 3.5, 3.4, 2.4, 3.8 & 3.4[p]	Post-kill: carcass, 4.6[a] Post-kill: carcass, 4.3[g] Post-stun: carcass, 5.93 & 5.36[h] Post-kill: neck skin, 4.2[m]
Scalding	Post-scald: carcass, 4.67[a] Post-scald: neck skin, 6.87 & 7.28[c] Post-scald: carcass, 5[g] Post-scald: breast skin, 5.76[j] Post-scald: carcass, 5.09 & 4.45[l] Post-scald rinse: carcass, 4.57[l] Post-scald: neck skin, 5.5, 5.7, 4.7, 5.6 & 5.9[p]	Post-scald: neck skin, (C) 4.76 & 5.01[c] Post-scald: neck skin, (E) 5.04 & 4.76[c] Post-scald: carcass, (C) 2.9[g] Post-scald: breast skin, (E) 3.36[j] Post-scald: carcass, (C) 2.56 & 1.8[l] Post-scald rinse: carcass, (C) 1.91[l] Post-scald: neck skin, (C) 2.8, 3.5, 2, 3.4 & 3.4[p]	Post-scald: carcass, 4.57[a] Post-scald: carcass, 2.1[g] Post-scald: carcass, 2.27 & 1.55[l] Post-scald rinse: carcass, 1.65[l]
Defeathering	Post-pick: neck	Post-pick: neck skin,	Post-pick:

	skin, 5.98 & 5.87[c] Post-pick: carcass, 5[g] Post-pick: carcass, 4.1 & 3.95[h] Post-pick: breast skin, 4.15[j] Post-pick: neck skin, 7 & 7.2[m] Pre-NYW: carcass, ~ 4.6 & 4.5[o] Post-NYW: carcass, ~ 4.1 & 4.1[o] Post-pick: neck skin, 5, 5.5, 4.6, 4.4 & 4.8[p]	(C) 4.53 & 4.27[c] Post-pick: neck skin, (E) 4.34 & 4.09[c] Post-pick: carcass, (C) 3.4[g] Post-pick: carcass, (C) 3.25 & 2.99[h] Post-pick: breast skin, (E) 2.54[j] Post-pick: neck skin, (E) 5.5 & 5.6[m] Post-pick: neck skin, (C) 5.4 & 5.6[m] Pre-NYW: carcass, (C) ~ 3.7 & 2.9[o] Post-NYW: carcass, (C) ~ 3.2 & 2.8[o] Post-pick: neck skin, (C) 3.3, 3.8, 2.2, 2.8 & 3[p]	carcass, 2.8[g] Post-pick: carcass, 3.02 & 2.8[h] Post-pick: neck skin, 4.9 & 4.8[m] Pre-NYW: carcass, ~ 3.4 & 2.6[o] Post-NYW: carcass, ~ 2.8 & 2.5[o]
Evisceration	Pre-salvage: carcass, 4.63[a] Post-salvage: carcass, 4.53[a] Post-evisceration: neck skin, 5.76 & 5.69[c] Pre-evisceration: carcass, 4.48[d] Post-evisceration: carcass, 3.81[d] Post-evisceration: carcass, 4.5[g] Pre-evisceration: carcass, 3.98[i] Post-evisceration: carcass, 4.11 & 4.39[l] Post-evisceration: neck skin, 7.2 & 7.2[m] Post-evisceration wash: carcass, ~ 4[o] Post-evisceration: neck skin, 5, 5.2,	Post-evisceration: neck skin, (C) 4.6 & 4.42[c] Post-evisceration: neck skin, (E) 4.55 & 3.90[c] Pre-evisceration: carcass, (C) 3.91[d] Post-evisceration: carcass, (C) 3.27[d] Post-evisceration: carcass, (C) 3.1[g] Pre-evisceration: carcass, (E) 3.22[i] Post-evisceration: carcass, (C) 2.71 & 3.03[l] Post-evisceration: neck skin, (E) 5.3 & 5.4[m] Post-evisceration: neck skin, (C) 5.2 & 5.7[m] Post-evisceration wash: carcass, (C) ~ 3.1[o]	Pre-salvage: carcass, 4.52[a] Post-salvage: carcass, 4.44[a] Pre-evisceration: carcass, 3.74[d] Post-evisceration: carcass, 3.08[d] Post-evisceration: carcass, 2.2[g] Pre-evisceration: carcass, 2.72[i] Post-evisceration: carcass, 2.47 & 2.82[l] Post-evisceration: neck skin, 4.9 & 5[m] Post-evisceration wash: carcass, ~ 2.7[o] Pre-evisceration: carcass, 2.09[q]

	4.9, 4.6 & 4.4[p] Pre-evisceration: carcass, 3.73[q]	Post-evisceration: neck skin, (C) 3.5, 3.9, 2.7, 3.4 & 3.1[p] Pre-evisceration: carcass, (E) 2.7[q]	
Carcass washers	Post-SW: neck skin, 5.1 & 4.95[c] Post-IOBW: carcass, 3.6[g] Pre-IOBW: carcass, 3.8, 5 & 4.4[k] Post-IOBW: carcass, 3.4, 4.7 & 3.8[k] Post-SW: neck skin, 6 & 6.3[m] Post-IOBW: carcass, ~ 3.3 & 3.6[o] Post-SW: neck skin, 4.6, 4.8, 4.4, 4.4 & 4.6[p]	Post-SW: neck skin, (C) 3.97 & 3.75[c] Post-SW: neck skin, (E) 3.77 & 3.67[c] Post-IOBW: carcass, (C) 2.2[g] Pre-IOBW: carcass, (C) 2.6, 3.7 & 3.4[k] Post-IOBW: carcass, (C) 2.4, 3.1 & 2.8[k] Post-SW: neck skin, (E) 5.2 & 5.2[m] Post-SW: neck skin, (C) 5.1 & 5.2[m] Post-IOBW: carcass, (C) ~ 2.2 & 2.5[o] Post-SW: neck skin, (C) 3.4, 3.6, 2.3, 3.1 & 3.1[p]	Pre-IOBW: carcass, 2.51 & 2.76[b] Post-IOBW: carcass, 1.45 & 2.80[b] Post-IOBW: carcass, 1.5[g] Pre-IOBW: carcass, 2.2, 3.3 & 3.1[k] Post-IOBW: carcass, 2.1, 2.7 & 2.4[k] Post-SW: neck skin, 1.9 & 2[m] Post-IOBW: carcass, ~ 1.9 & 2[o]
Carcass reprocessing	Pre-Cecure®: carcass, 4.6[a] Post- Cecure®: carcass, 2.2[a] Post-ASC: carcass, ~ 3.1[o] Post-TSP: carcass, ~ 3.2 & 3.3[o]	Post-ASC: carcass, (C) ~ 1.8[o] Post-TSP: carcass, (C) ~ 1.7 & 1.4[o]	Pre-Cecure®: carcass, 4.5[a] Post- Cecure®: carcass, 1.96[a] Post-ASC: carcass, ~ 1.4[o] Post-TSP: carcass, ~ 1.4 & 1.1[o]
Carcass chilling	Pre-chill: carcass, 1.97[a] Post-chill: carcass, 1.95[a] Post-chill: neck skin, 5.18 & 5.13[c] Post-chill: carcass, 3.23[d] Post-chill: carcass, 3.38[f] Post-airchill: carcass, 3.31[f] Post-chill:	Post-chill: neck skin, (C) 3.99 & 3.92[c] Post-chill: neck skin, (E) 3.81 & 3.91[c] Post-airchill: carcass, (C) 2.59[d] Pre-chill: carcass, (C) 3.9[e] Post-chill: carcass, (C) 2.6[e] Post-chill: carcass, (C) 1.72[f] Post-airchill: carcass,	Pre-chill: carcass, 1.84[a] Post-chill: carcass, 1.83[a] Post-chill: carcass, 1.22 & 1.74[b] Post-airchill: carcass, 2.2[d] Pre-chill: carcass, 3.2[e] Post-chill: carcass, 1.8[e]

	carcass, 2.9[g] Pre-chill: carcass, 3.2[i] Post-chill: carcass, 2.51[i] Pre-chill: breast skin, 3.91[j] Post-chill: breast skin, 3.71[j] Post-chill: carcass, 3.59 & 4.01[l] Post-airchill: neck skin, 6 & 6.1[m] Pre-chill: carcass, ~ 4.2[n] Post-chill: carcass, ~ 3.25[n] Post-Cl_2 chill: carcass, ~ 2.7 & 2.9[o] Post-ASC/Cl_2 chill: carcass, ~ 2.1[o] Post-chill: neck skin, 4.4, 5.1, 3.6, 4.4 & 4.7[p] Pre-chill: carcass, 3.18[q] Post-chill: carcass, 2.87[q]	(C) 1.97[f] Post-chill: carcass, (C) 1.9[g] Pre-chill: carcass, (E) 2.57[i] Post-chill: carcass, (E) 1.75[i] Pre-chill: breast skin, (E) 2.64[j] Post-chill: breast skin, (E) 2.51[j] Post-chill: carcass, (C) 2.25 & 2.6[l] Post-airchill: neck skin, (E) 5.3 & 5[m] Post-airchill: neck skin, (C) 4.3 & 5[m] Post-Cl_2 chill: carcass, (C) ~ 1.2 & 1.3[o] Post-ASC/Cl_2 chill: carcass, (C) ~ 0.8[o] Post-chill: neck skin, (C) 2.8, 3.7, 3 & 3[p] Pre-chill: carcass, (E) 2.25[q] Post-chill: carcass, (E) 1.56[q]	Post-chill: carcass, 1.17[f] Post-airchill: carcass, 1.43[f] Post-chill: carcass, 1.1[g] Pre-chill: carcass, 2.04[i] Post-chill: carcass, 1.2[i] Post-chill: carcass, 2.09 & 2.36[l] Post-airchill: neck skin, 4.4 & 4.1[m] Pre-chill: carcass, ~ 2.4[n] Post-chill: carcass, ~ 1.2[n] Post-Cl_2 chill: carcass, ~ 0.8 & 1[o] Post-ASC/Cl_2 chill: carcass, ~ 0.6[o] Pre-chill: carcass, 1.61[q] Post-chill: carcass, 0.89[q]
Post chilling treatment			Post-chill ASC: carcass, 0[b]
Packaging/retail	Post-pack: neck skin, 6.4 & 6.5[m] Post-pack: neck skin, 4.5, 5.3, 4.4 & 5.1[p]	Post-pack: neck skin, (E) 5.4 & 5[m] Post-pack: neck skin, (C) 5 & 4.8[m] Post-pack: neck skin, (C) 2.7, 3.8, 3.3 & 3.4[p]	Post-pack: neck skin, 4 & 3.9[m]

[a]Russell (2005)
[b]Oyarzabal and others (2004)
[c]Göksoy and others (2004) *The unit is log_{10} cfu/g of neck skin
[d]Fluckey and others (2003)
[e]Northcutt and others (2003a)
[f]Sanchez and others (2002)

[g]Berrang and Dickens (2000)
[h]Buhr and others (2000)
[i]James and others (1992a)
[j]Lillard (1989) *The unit is $\log_{10}$ cfu/g of breast skin
[k]Northcutt and others (2003b)
[l]Waldroup and others (1993b)
[m]Abu-Ruwaida and others (1994) *The unit is $\log_{10}$ cfu/g of neck skin
[n]Bilgili and others (2002)
[o]Stopforth and others (2007)
[p]Mead and others (1993) *The unit is $\log_{10}$ cfu/g of neck skin
[q]James and others (1992b)
General format: Stage: sample type, count
Abbreviation: IOBW=Inside outside bird washer; SW=Spray washer; NYW=New York Wash (Chlorine Wash after defeathering); ASC=Acidified sodium chlorite; TSP=Trisodium phosphate
*Carcass=carcass rinse; post-chill=post-immersion chlorine chiller; All washes are chlorine unless indicated

Appendix J. *Salmonella spp.* incidence and populations measured in poultry production and processing by various researchers

Stages of Processing	Incidence	Populations (log_{10} MPN/ml)
Farm	1-day before processing: cecum, 22%[c] Pre-8h feed withdrawal: crop, 0%, 0%, 0%, 0% & 10%[g] Post-8h feed withdrawal: crop, 7.5%, 10%, 5%, 2.6% & 30%[g] Pre-8h feed withdrawal: cecum, 0% & 2.5%[g] Post-8h feed withdrawal: cecum, 7.5% & 17.5%[g]	1-day before processing: litter drag swab, 1.67[c]
Transportation/initial level entering plant	Post-kill: carcass, 92.3 %[a] Post-kill: neck skin, 40% & 33.3%[b] Post-stun: carcass, 59.4% & 43.8%[f] Post-kill: whole carcass swab, 7%[m]	
Scalding	Post-scald: carcass, 90.4%[a] Post-scald: neck skin, 33.3% & 33.3%[b] Post-scald: carcass, 11.3% & 1.3%[j] Post-scald rinse: carcass, 6.3%[j]	
Defeathering	Post-pick: neck skin, 60% & 40%[b] Post-pick: carcass, 59.4% & 40.6%[f] Post-pick: carcass, 23%[h] Post-pick: whole carcass swab, 16%[m]	Post-pick: carcass: 1.2[e]
Evisceration	Pre-salvage: carcass, 84.6%[a] Post-salvage: carcass, 75%[a] Post-evisceration: neck skin, 26.6% & 33.3%[b] Pre-evisceration: carcass, 33%[i]	Pre-evisceration: carcass, ~1.35[c] Post-evisceration: carcass, ~1.18[c]

	Post-evisceration: carcass, 27.5% & 28.8%[j] Pre-evisceration: whole carcass swab, 5%[m] Post-evisceration: whole carcass swab, 11%[m] Pre-evisceration: carcass, 24%[p]	
Carcass washers	Post-SW: neck skin, 26.6% & 20%[b] Post-SW (H_2O): whole carcass swab, 1%[m] Pre-IOBW: carcass, 95%[o]	Pre-IOBW: carcass, 1.56[o]
Carcass reprocessing	Pre-Cecure®: carcass, 84.6%[a] Post- Cecure®: carcass, 17.3%[a]	
Carcass chilling	Pre-chill: carcass, 15.4%[a] Post-chill: carcass, 13.5%[a] Post-chill: neck skin, 33.3% & 26.6%[b] Post-chill: carcass, 24.7%[d] Post-airchill: carcass, 18.7%[d] Pre-chill: carcass, 20%[h] Post-chill: carcass, 19%[h] Pre-chill: carcass, 43%[i] Post-chill: carcass, 46%[i] Post-chill: carcass, 27.5% & 30.4%[j] Pre-chill: carcass enrichment, 88.4%[k] Post-chill: carcass enrichment, 84.1%[k] Pre-chill: carcass, 20.7%[l] Post-chill: carcass, 5.7%[l] Pre-chill: whole carcass swab, 10%[m] Post-chill: whole carcass swab, 16%[m] Pre-chill: carcass swab, 26% & 20%[n] Post-chill: carcass swab, 6.4% & 13.7%[n] Pre-chill: carcass, 100%[o] Post-chill: carcass, 41.7%[o]	Post-airchill: carcass, ~0.94[c] Pre-chill: carcass, 0.75[o] Post-chill: carcass, -1.29[o]

	Pre-chill: carcass, 28%[p] Post-chill: carcass, 49%[p]	

[a]Russell (2005)
[b]Göksoy and others (2004) *The unit is log_{10} cfu/g of neck skin
[c]Fluckey and others (2003)
[d]Sanchez and others (2002)
[e]Cason and others (2000)
[f]Buhr and others (2000)
[g]Corrier and others (1999)
[h]Cason and others (1997)
[i]James and others (1992a)
[j]Waldroup and others (1993b)
[k]Parveen and others (2007)
[l]Bilgili and others (2002)
[m]Nde and others (2006)
[n]Logue and others (2003)
[o]Brichta-Harhay and others (2007)
[p]James and others (1992b)
General format: Stage: sample type, count
Abbreviation: MPN=Most probable number; IOBW=Inside outside bird washer; SW=Spray washer; ASC=Acidified sodium chlorite
*Carcass=carcass rinse; post-chill=post-immersion chlorine chiller; All washes are chlorine unless indicated

Appendix K. Average coliform, *Salmonella spp.* and *Campylobacter spp.* counts of carcass rinses taken from different stages of a poultry processing plant

	Coliform Counts (log_{10} cfu/ml±SE) n=5	*Salmonella spp.* Counts (log_{10} cfu/ml±SE) n=5	*Campylobacter spp.* Counts (log_{10} cfu/ml±SE) n=5	Biochemical Identification of Presumptive *Campylobacter*[a]
Receiving	5.47±0.20	0.56±0.27	5.29±0.08	I: 5 of 5 negative II: 5 of 5 negative III: 3 of 5 *C. jejuni* & 2 of 5 *C. coli* IV: 1 of 5 *C. jejuni* V: 1 of 5 *C. lari*
Post-scald	4.53±0.19	0.72±0.30	3.46±0.07	I: 5 of 5 *C. lari* II: 5 of 5 negative III: 5 of 5 negative IV: 1 of 5 *C. jejuni* V: 1 of 5 *C. coli*
Post-pick	3.64±0.30	0.30±0.28	2.75±0.26	I: 5 of 5 negative II: 5 of 5 negative III: 3 of 5 *C. lari* IV: 3 of 5 *C. lari* V: 5 of 5 negative
Rehang/pre-evisceration	2.95±0.16	undetectable	2.73±0.17	I: 5 of 5 negative II: 1 of 5 *C. jejuni* III: 5 of 5 negative IV: 5 of 5 negative V: 5 of 5 negative
Post-evisceration	3.71±0.25	undetectable	2.25±0.39	I: 5 of 5 negative II: 3 of 5 *C. jejuni* III: 5 of 5 negative IV: 5 of 5 negative V: 2 of 5 *C. jejuni*
Post-IOBW	3.07±0.23	undetectable	1.76±0.21	I: 2 of 5 *C. jejuni* & 1 of 5 *C. coli* II: 5 of 5 negative III: 5 of 5 negative IV: 1 of 5 *C. jejuni* V: 5 of 5 negative
Pre-SANOVA®	2.77±0.17	undetectable	1.53±0.31	I: 5 of 5 negative II: 5 of 5 negative III: 2 of 5 *C. jejuni* IV: 5 of 5 negative V: 2 of 5 *C. jejuni*
Post-SANOVA®	1.35±0.37	undetectable	0.41±0.17	I: 5 of 5 negative II: 5 of 5 negative

				III: 5 of 5 negative IV: 5 of 5 negative V: 5 of 5 negative
Post-chill	0.38±0.28	undetectable	0.61±0.27	I: Not tested II: Not tested III: Not tested IV: 5 of 5 negative V: Not tested

Each coliform, *Salmonella spp.* and *Campylobacter spp.* count is an average of duplicates
[a]Five presumptive colonies were taken from mCampy-Cefex media and tested biochemically for *Campylobacter spp.* Oxidase test serves as an initial screening test. All oxidase negative colonies are designated as negative (not *C. jejuni, C. coli* and *C. lari*). Oxidase positive colonies are tested against hippurate hydrolysis, naladixic acid resistance and cephalothin resistance.

Appendix L. Results of t-test between two means for coliform, *Salmonella spp.,* and *Campylobacter spp.* counts of carcass rinse samples taken from different stages of a poultry processing line

	Mean Difference (log_{10} cfu/ml) n=10	T-value	P-value	Interpretation
Coliform Counts				
Receiving – Post-scald	0.9484	3.39	0.0094	Significant
Post-scald – Post-pick	0.8834	2.38	0.0443	Significant
Rehang – Post-evisceration	-0.7680	-2.49	0.0378	Significant
Post-evisceration – Post-IOBW	0.6413	1.79	0.1110	Not significant
Pre-SANOVA™ - Post-SANOVA™	1.4189	3.87	0.0048	Significant
Post-SANOVA™ - Post-chill	0.9737	2.31	0.0499	Significant
Salmonella spp. Counts				
Receiving – Post-scald	-0.1600	-0.41	0.6956	Not significant
Post-scald – Post-pick	0.4250	1.01	0.3417	Not significant
Campylobacter spp. Counts				
Receiving – Post-scald	1.8321	16.43	<0.0001	Significant
Post-scald – Post-pick	0.7068	2.89	0.0201	Significant
Rehang – Post-evisceration	0.4861	3.33	0.0104	Significant
Post-evisceration – Post-IOBW	0.4866	2.19	0.0600	Not significant
Pre-SANOVA™ - Post-SANOVA™	1.1204	4.74	0.0015	Significant
Post-SANOVA™ - Post-chill	-02030	-0.64	0.5390	Not significant

$\alpha = 0.05$

Appendix M. Average total aerobic and coliform counts of carcass rinses taken from pre-OLR, post-OLR, and post-chill stages of a processing plant using two types of OLR antimicrobial solutions

		Total Aerobic Count (log_{10} cfu/ml±SE)			Coliform Count (log_{10} cfu/ml±)		
Day	Line	Pre-OLR n=10	Post-OLR n=10	Post-chill n=10	Pre-OLR n=10	Post-OLR n=10	Post-chill n=10
1	1	4.12± 0.16	3.80± 0.19	1.67± 0.18[a]	3.40± 0.15	2.65± 0.18	0.50± 0.21[a]
2	1	3.85± 0.08	2.90± 0.10	1.92± 0.15	2.38± 0.10	0.52± 0.17	0.06± 0.06
3	1	4.09± 0.12	3.13± 0.12	1.55± 0.16	2.91± 0.17	0.36± 0.12	0.90± 0.06
4	1	4.15± 0.12	3.37± 0.18	1.88± 0.14	2.95± 0.18	1.09± 0.24	0.59± 0.18
Line 1 Total n=40		4.05± 0.06	3.30± 0.09	1.76± 0.08[b]	2.91± 0.09	1.15± 0.17	0.31± 0.08[b]
1	2	4.50± 0.15	3.92± 0.11	1.55± 0.23	3.93± 0.18	2.98± 0.16	0.09± 0.05
2	2	3.78± 0.09	3.93± 0.15	1.57± 0.15	2.40± 0.15	2.93± 0.24	0.00± 0.00
3	2	4.02± 0.05	3.67± 0.08	1.73± 0.08	2.72± 0.10	2.34± 0.12	0.07± 0.07
4	2	3.93± 0.10	3.59± 0.14	1.81± 0.10	2.79± 0.13	2.05± 0.26	0.40± 0.09
Line 2 Total n=40		4.06± 0.07	3.78± 0.06	1.66± 0.07	2.96± 0.12	2.56± 0.12	0.14± 0.04
SANOVA™ Total n=80		4.06± 0.05	3.54± 0.06	1.71± 0.05[c]	2.93± 0.07	1.86± 0.13	0.22± 0.04[c]
5	1	3.75± 0.08	3.25± 0.15	1.64± 0.23	2.71± 0.11	0.56± 0.14	0.55± 0.22
6	1	4.61± 0.20	3.31± 0.08	1.72± 0.16	3.37± 0.21	0.92± 0.13	0.37± 0.12
7	1	4.07± 0.06	3.69± 0.09	1.71± 0.15	2.72± 0.06	1.68± 0.13	0.44± 0.12
8	1	4.32± 0.15[a]	3.05± 0.08	2.15± 0.08	3.19± 0.16	0.78± 0.16	0.74± 0.12
Line 1 Total n=40		4.18± 0.08[b]	3.33± 0.06	1.81± 0.09	3.00± 0.08	0.98± 0.10	0.52± 0.08
5	2	3.89± 0.10	3.91± 0.17	1.79± 0.17	2.94± 0.15	2.82± 0.18	0.37± 0.15
6	2	5.12± 0.23	4.45± 0.15	1.29± 0.23	4.32± 0.21	3.59± 0.19	0.46± 0.16
7	2	3.96± 0.10	4.17± 0.15	1.60± 0.13	2.97± 0.14	3.14± 0.20	0.34± 0.14

8	2	4.46± 0.13	3.93± 0.13	2.12± 0.23	3.28± 0.18	2.58± 0.20	0.09± 0.06
Line 2 Total n=40		4.36± 0.11	4.11± 0.08	1.70± 0.11	3.38± 0.12	3.03± 0.11	0.32± 0.07
PERASAFE® Total n=80		4.27± 0.07[c]	3.72± 0.07	1.75± 0.07	3.19± 0.08	2.01± 0.14	0.42± 0.05

[a]n=9 instead of n=10
[b]n=39 instead of n=40
[c]n=79 instead of n=80

Appendix N. Comparison of total aerobic and coliform count reductions between two antimicrobial solutions

	Mean Difference (log_{10} cfu/ml)	T-value	P-value	Interpretation
Line 1 (TAC) n=160				
($Pre\text{-}OLR_S$ – $Post\text{-}OLR_S$) – ($Pre\text{-}OLR_P$ – $Post\text{-}OLR_P$)[a]	-0.05492867	-0.73	0.4650	Not Significant
($Pre\text{-}OLR_S$ – $Post\text{-}chill_S$) – ($Pre\text{-}OLR_P$ – $Post\text{-}chill_P$)[b]	-0.04163848	-0.53	0.5956	Not Significant
($Post\text{-}OLR_S$ – $Post\text{-}chill_S$) – ($Post\text{-}OLR_P$ – $Post\text{-}chill_P$)[a]	0.01329018	0.16	0.8693	Not Significant
Line 2 (TAC) n=160				
($Pre\text{-}OLR_S$ – $Post\text{-}OLR_S$) – ($Pre\text{-}OLR_P$ – $Post\text{-}OLR_P$)	0.02024509	0.25	0.8033	Not Significant
($Pre\text{-}OLR_S$ – $Post\text{-}chill_S$) – ($Pre\text{-}OLR_P$ – $Post\text{-}chill_P$)	-0.13248937	-0.14	0.1431	Not Significant
($Post\text{-}OLR_S$ – $Post\text{-}chill_S$) – ($Post\text{-}OLR_P$ – $Post\text{-}chill_P$)	-0.15273445	-1.86	0.0654	Not Significant
Both Lines (TAC) n=320				
($Pre\text{-}OLR_S$ – $Post\text{-}OLR_S$) – ($Pre\text{-}OLR_P$ – $Post\text{-}OLR_P$)[c]	-0.01674464	-0.29	0.7711	Not Significant
($Pre\text{-}OLR_S$ – $Post\text{-}chill_S$) – ($Pre\text{-}OLR_P$ – $Post\text{-}chill_P$)[d]	-0.08735326	-1.46	0.1448	Not Significant
($Post\text{-}OLR_S$ – $Post\text{-}chill_S$) – ($Post\text{-}OLR_P$ – $Post\text{-}chill_P$)[c]	-0.06858802	-1.12	0.2639	Not Significant
Line 1 (Coliform Count) n=160				
($Pre\text{-}OLR_S$ – $Post\text{-}OLR_S$) – ($Pre\text{-}OLR_P$ – $Post\text{-}OLR_P$)	-0.12774459	-1.10	0.2743	Not Significant
($Pre\text{-}OLR_S$ – $Post\text{-}chill_S$) – ($Pre\text{-}OLR_P$ – $Post\text{-}chill_P$)[a]	0.06487419	0.78	0.4390	Not Significant
($Post\text{-}OLR_S$ – $Post\text{-}chill_S$) – ($Post\text{-}OLR_P$ – $Post\text{-}chill_P$)[a]	0.19261878	1.72	0.0874	Not Significant
Line 2 (Coliform Count)				

n=160				
$(Pre\text{-}OLR_S - Post\text{-}OLR_S) - (Pre\text{-}OLR_P - Post\text{-}OLR_P)$	0.01870484	0.16	0.8722	Not Significant
$(Pre\text{-}OLR_S - Post\text{-}chill_S) - (Pre\text{-}OLR_P - Post\text{-}chill_P)$	-0.12004058	-1.30	0.1961	Not Significant
$(Post\text{-}OLR_S - Post\text{-}chill_S) - (Post\text{-}OLR_P - Post\text{-}chill_P)$	-0.13874541	-1.56	0.1219	Not Significant
Both Lines (Coliform Count) n=320				
$(Pre\text{-}OLR_S - Post\text{-}OLR_S) - (Pre\text{-}OLR_P - Post\text{-}OLR_P)$	-0.06077277	-0.65	0.5162	Not Significant
$(Pre\text{-}OLR_S - Post\text{-}chill_S) - (Pre\text{-}OLR_P - Post\text{-}chill_P)$[c]	-0.02700972	-0.43	0.6705	Not Significant
$(Post\text{-}OLR_S - Post\text{-}chill_S) - (Post\text{-}OLR_P - Post\text{-}chill_P)$[c]	0.02992496	0.33	0.7407	Not Significant

[a]n=159 instead of n=160
[b]n=158 instead of n=160
[c]n=319 instead of n=320
[d]n=318 instead of n=320
$\alpha = 0.05$
Abbreviation: TAC=Total aerobic count; OLR=Online reprocessing; Subscript S=SANOVA™; Subscript P=PERASAFE®

Appendix O. GLM table for total aerobic and coliform counts in carcass rinses taken at pre-OLR stage of poultry processing

Source	Mean Square	F-value	P-value	Interpretation
Total Aerobic Count				
Line	0.32401087	1.97	0.1625	Not significant
Day	2.42488875	14.75	<0.0001	Significant
Line*Day	0.32046233	1.95	0.0660	Not significant
Coliform Count				
Line	1.82951114	7.66	0.0064	Significant
Day	4.76338507	19.95	<0.0001	Significant
Line*Day	0.71360331	2.99	0.0059	Significant

α=0.05

Appendix P. Least mean squares of total aerobic and coliform counts in carcass rinses taken at pre-OLR stage of poultry processing based on line and day of collection

	Line	log_{10} cfu/ml±SE	Day	log_{10} cfu/ml±SE	Line*Day	log_{10} cfu/ml±SE
Total Aerobic Count	1	4.12±0.05	1	4.31±0.09	1*1	4.12±0.13
	2	4.21±0.05	2	3.81±0.09	1*2	3.85±0.13
			3	4.06±0.09	1*3	4.09±0.13
			4	4.04±0.09	1*4	4.15±0.13
			5	3.82±0.09	1*5	3.75±0.13
			6	4.86±0.09	1*6	4.61±0.13
			7	4.02±0.09	1*7	4.07±0.13
			8	4.39±0.09	1*8	4.32±0.13
					2*1	4.50±0.13
					2*2	3.78±0.13
					2*3	4.02±0.13
					2*4	3.93±0.13
					2*5	3.89±0.13
					2*6	5.12±0.13
					2*7	3.96±0.13
					2*8	4.46±0.13
Coliform Count	1	2.95±0.05	1	3.67±0.11	1*1	3.40±0.15
	2	3.17±0.05	2	2.39±0.11	1*2	2.38±0.15
			3	2.81±0.11	1*3	2.91±0.15
			4	2.87±0.11	1*4	2.95±0.15
			5	2.83±0.11	1*5	2.71±0.15
			6	2.85±0.11	1*6	3.37±0.15
			7	2.84±0.11	1*7	2.72±0.15
			8	3.23±0.11	1*8	3.19±0.15
					2*1	3.93±0.15
					2*2	2.40±0.15
					2*3	2.72±0.15
					2*4	2.79±0.15
					2*5	2.94±0.15
					2*6	4.32±0.15
					2*7	2.97±0.15
					2*8	3.28±0.15

Different flocks are slaughtered on different days.

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