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System Dynamics Simulation Model of Salmonella Contamination of Broiler Carcasses in the Chill Tank of a Poultry Processing Plant

Karen Dazo Galarneau

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By

Karen Dazo Galarneau

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By

Karen Dazo Galarneau

Approved:

Robert W. Wills
(Major Professor)

Sherman W. Jack
(Co-Major Professor)

R. Hartford Bailey
(Committee Member)

Carla L. Huston
(Committee Member)

Filip Suminto D. To
(Committee Member)

Mark L. Lawrence
Associate Dean
College of Veterinary Medicine

Name: Karen Dazo Galarneau

Date of Degree: December 14, 2013

Institution: Mississippi State University

Major Field: Veterinary Medical Sciences

Major Professor: Robert W. Wills

Title of Study: System dynamics simulation model of *Salmonella* contamination of broiler carcasses in the chill tank of the poultry processing plant

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Candidate for Degree of Doctor of Philosophy

Salmonella has been studied and researched for more than a hundred years and yet it remains a problem for human and animal health. The goal of this dissertation was to apply the systems thinking approach to *Salmonella* contamination and develop a System Dynamics (SD) simulation model for *Salmonella* contamination in the chill tank of a poultry processing plant. But first the appropriate carcass rinse sampling method that would not impact on the resulting *Salmonella* contamination status of the broiler carcass was studied. Kappa agreement analysis was used to evaluate three sampling methods. The adjacent rinse method was found to be the best method.

In the absence of actual data, literature data was used to develop a literature-based SD simulation model of *Salmonella* contamination of broiler carcasses in the chill tank. The literature-based SD model is the first application of system dynamics simulation modeling in the poultry-processing field. The model was able to show and simulate the dynamic and non-linear interrelationships between parameters, namely pH, chlorine (Cl) level, water flow and turbidity.

Actual data collection was done using a specially designed apparatus that recorded the time, temperature, pH, chlorine, water flow and turbidity in the chill tank as carcass rinse samples were collected. Linear regression analysis was used to identify the statistically significant models for relationships between the parameters in the chill tank.

Finally, the data was analyzed using logistic regression to determine the association between the parameters in the chill tank and the occurrence of *Salmonella* in carcasses exiting the chill tank. These results were used to develop a data-based SD model. The data-based model was then validated using the validity tests proposed by various authors for SD simulation models and found to be a valid model. The developed model offers a fresh perspective to the problem of *Salmonella* contamination- to view it as a system of factors that are interrelated and have a feedback mechanism, rather than the traditional concept of linear causation. The developed model is a powerful cost-efficient tool for testing interventions for reducing *Salmonella* contamination in the poultry processing plant.

DEDICATION

This dissertation is dedicated to my loving mother, Lilia Cichon Dazo, my wonderful husband and better half, Scott Lewis Galarneau, and to the memory of my departed father, P/Captain Orencio Ticao Dazo.

Mommy, you taught me how to pray, to read, and to be kind to others. You gave me the gift of learning, music, poetry and the arts. You are my salient source of inspiration, encouragement and strength. Thank you for being the greatest Mom in the world!

Honey, your faithful love and unwavering support has carried me through to the fulfillment of this dream. I am looking forward to sharing more journeys and realizing more dreams with you. (lummmmm).

Daddy, I know you can see me from heaven. Thank you for being the best father we could ever have. Thank you for always encouraging me to strive for excellence and for your leadership by example.

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CHAPTER I

REVIEW OF LITERATURE

Contemporary importance of *Salmonella* contamination

Salmonella contamination of raw poultry meat is a concern because of its public health implications (Mead et al., 1999). The disease salmonellosis is a serious illness and can be fatal to immune-compromised, or extremely old or extremely young people. Processed broiler carcasses are frequently contaminated with *Salmonella* and other micro-organisms of public health importance (Panisello et al., 2000). For the past fifteen years, *Salmonella* infections have been the most common foodborne infection and cause of hospitalization and death tracked in the FoodNet surveillance program (CDC, 2011). *Salmonella typhimurium* and *enteritidis* are the two most common serovars found in poultry and poultry products (Gast, 2003) A similar pattern, but with the addition of Newport, has been observed in human salmonellosis (CDC, 2011). According to the Centers for Disease Prevention and Control (CDC), *Salmonella* infection was the most common bacterial foodborne infection reported in the United States in 2010 and among the isolates, the most common serotypes were Enteritidis (22%), Newport (14%) and Typhimurium (13%) (CDC, 2011).

Salmonella contamination of broiler carcasses poses a public health hazard to the consumers and continues to be a problem for the broiler industry in the 21st century. For a long time now it has been known that the handling and consumption of contaminated

poultry meat is a major cause of food-borne illness (Bryan, 1980). It was estimated that *Salmonella* spp. cause 1.2 million out of around 9.4 million foodborne illnesses each year in the United States (Scallan et al., 2011). The annual total direct medical expenditures due to food-borne *Salmonella* infections in the U.S. was estimated at $365 million (USDA, 2010b).

Prevention of *Salmonella* contamination is one of the priorities established by the U.S. Department of Agriculture (USDA) in its goal of enhancing protection and safety of the nation's agriculture and food supply (USDA, 2005). The reduction of *Salmonella* prevalence in broiler chickens is one of the annual performance indicators used by the Food Safety and Inspection Services (FSIS) of the USDA (USDA, 2005). Compliance to Hazard Analysis Critical Control Points (HACCP) requirement is determined by measuring *Salmonella* prevalence in the broiler carcasses as they exit the chill tank. In 2005, the USDA issued new procedures with regards to *Salmonella* testing in food safety (USDA, 2005), which triggered an in-depth review of the plant's operations, operator's training, and HACCP plans as soon as any series of the tests got declared as below standard. Previously, companies had to have two consecutive failures of testing sets before the USDA would conduct an in-depth review of the plant's operations and HACCP plans. However, the problem of *Salmonella* contamination has remained a challenge for the poultry companies, the regulatory agencies and the consuming public. In June of 2011, the USDA Food Safety and Inspection Service (FSIS) in Washington, DC, issued FSIS Notice 31-11 "New performance standards for *Salmonella* and *Campylobacter* in chilled carcasses in young chicken and turkey slaughter establishments" (USDA, 2011b). Under these standards for *Salmonella*, the

establishment could have no more than five positive samples for *Salmonella* in a 51-sample set for young chickens and no more than four positive samples in a 56-sample set for turkeys. This means that for young chickens, a set is made up of 51 whole carcass rinse samples, and the limit is 5 samples to be found *Salmonella* positive. For the *Campylobacter* standards, the establishment could not have more than eight positive samples in a 51 sample set for young chickens and no more than three positive samples in a 56 sample set for turkeys. In comparison with earlier regulations, the new guidelines required a higher level of compliance.

Sources of contamination

During processing, the chicken's crop and ceca are major sources for broiler carcass contamination (Amit-Romach et al., 2004; Ramirez et al., 1997). The skin, feathers and feet of the carcasses can also be a source of contamination or cross-contamination (Bryan and Doyle, 1995). *Salmonella* prevalence has been shown to increase significantly from pre-chill to post-chill due to cross-contamination between carcasses during chilling (James et al., 1992b). Brant et al. (1982) reported that workers in the processing plant can be carriers of the organism but infected live poultry is the most important source of *Salmonella*. They also reported that a higher percentage of contamination occurred when a *Salmonella* positive flock was processed.

Public health significance

Salmonella has been reported as the most commonly diagnosed bacterial causative agent of food-borne infections in humans in the U.S. (Chittick et al., 2006). Poultry and egg-containing products were implicated as the cause of majority of

Salmonella heidelberg outbreaks (Chittick et al., 2006). In addition, the emergence of multidrug-resistant *Salmonella* has been described as a major public health concern, particularly to ceftriaxone and ciprofloxacin, since these drugs are important in the treatment of salmonellosis in both adults and children (Chen et al., 2004). Improper handling and subsequent consumption of contaminated poultry meat are major contributors to food-borne illness in humans and has been a concern for decades, particularly when the meat is eaten raw, undercooked or re-contaminated and improperly stored following cooking (Bryan, 1980). It is important to note that FoodNet, a major food safety surveillance system, has reported that there has been no decline in the importance of *Salmonella* infections as the most common foodborne infection resulting in illness and mortality (CDC, 2011).

Pathogenesis

Salmonellae bacteria are commonly ingested along with contaminated foodstuffs. Some of these organisms survive the gastric acid in the stomach. When the bacteria reach the small intestines and make contact with the intestinal epithelial cells, they cause a phenomenon called "membrane ruffling" as well as other damage to the enterocyte cytoskeleton (Chen et al., 1996). Membrane ruffling allows the internalization of the bacteria into the cell through bacterial mediated endocytosis (Lesser et al., 2000).

Signs and symptoms of salmonellosis

Salmonellosis is the disease caused by the non-typhoidal *Salmonella* species such as *Salmonella typhimurium, S.enteritidis, S. heidelberg, S. dublin, S. senftenberg* and others. Clinical salmonellosis, which can be a food-borne infection, is characterized by

diarrhea, nausea, vomiting, fever, abdominal cramps, general body weakness and if untreated could lead to dehydration and even death in susceptible populations e.g., young, old, or immune-compromised patients (Gast, 2003). The disease in humans occurs 24-36 hours after ingestion and lasts from two to seven days. *Salmonella typhimurium* and *enteritidis* are the two most common serovars found in poultry and poultry products (Gast, 2003).

Kitchen hygiene

Good kitchen hygiene is important to prevent salmonellosis. Mishandling poultry during final preparation for human consumption can lead to infection. Otherwise, cooking would kill the salmonellae. Poor kitchen hygiene can lead to cross-contamination of other foods during preparation as well. Undercooking and contamination after the food has been cooked are the most common problems (Bryan, 1980).

History and classification of *Salmonella*

Salmonella bacteria were first isolated in the late 1800's by Daniel Salmon and Theobald Smith (Salmon and Smith, 1886). The first isolates were found in a pig infected with hog cholera hence it was initially named as *Vibrio choleraesuis*. However, it was later found not to be the causative agent of Hog Cholera. In 1900, this group of bacteria was named after Dr. Salmon by the French bacteriologist Leon Marcel Lignieres (Le Minor, 1981).

The genus *Salmonella* belongs to the family *Enterobacteriaceae*. The nomenclature of *Salmonella* that evolved through the years resulted in naming more than

2000 serovars and five subgenera (Wayne et al., 1987), a schematic which has been further updated. To date it is generally accepted that there are two species under the genus *Salmonella*, namely, *Salmonella enterica* and *Salmonella bongori* (Grimont, 2000). There are more than 2,400 motile and nonhost-adapted serotypes under *S. enterica* commonly called paratyphoid (PT) salmonellae, including *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhimurium. However, *S. enteritidis* or *S. typhimurium* and other traditional names are still used as convenient nomenclature for facilitating diagnostic and epidemiologic classification. Throughout this paper, the term "salmonellae" is the generic word used to refer to all *Salmonella* bacteria.

The paratyphoid salmonellae group

Paratyphoid (PT) salmonellae are gram-negative, straight rods that do not form spores (Gast, 2003). In addition to gram-staining, they also stain readily with methylene blue and carbolfuchsin (a mixture of phenol and a solution of fuchsin used as a stain). These bacteria are commonly peritrichous (having flagella around the surface of the bacterial cell) and motile. They are facultative anaerobes. They grow best at 37˚C but also grow over a range from 5˚C to 45˚C. *Salmonella* grow best at pH 7.0 but can grow within a range of pH from 4.0 to 9.0. They have simple nutritional requirements and will grow on most culture media that contain nitrogen and carbon. On agar media, *Salmonella* appear as round colonies that measure 2-4 mm in diameter, are slightly raised, glistening, and have smooth edges. Biochemically, PT salmonellae ferment sugars like glucose (producing acid as well as gas), dulcitol, mannitol, maltose and mucate (Holt et al., 1994). They are unable to ferment lactose, sucrose, malonate, or salicin. They produce hydrogen sulfide (H_2S), can use citrate as sole carbon source, are

able to reduce nitrates to nitrites, and can decarboxylate ornithine and lysine. Paratyphoid salmonellae do not hydrolyze urea or gelatin, and are negative for indole production (Holt et al., 1994).

Conventional methods of *Salmonella* isolation

The most commonly used selective broth media for isolating PT salmonellae are tetrathionate (TT) broth and Rappaport Vassiliadis (RV) broth (Gast, 2003). A combination of TT and RV has been proposed as a more effective method of *Salmonella* detection (Rybolt et al., 2004; Vassiliadis et al., 1978). A number of agar media are used for the isolation of PT salmonellae including Brilliant Green (BG) agar, XLD agar, XLT4 agar, bismuth sulfite agar, and Hektoen enteric agar (Gast, 2003).

Rapid detection methods

Some rapid detection methods for *Salmonella* include enzyme-linked immunosorbent assay (ELISA) (Wyatt et al., 1995), immunomagnetic bead ELISAs (Cudjoe et al., 1995) and dot immuno-binding assays (Charles et al., 1996). Molecular methods such as gene probes, plasmid analysis, and ribotyping have also been used for detection of *Salmonella* (Betts et al., 1995). Another molecular method is the polymerase chain reaction (PCR) BAX system. This system is one of the commercially available identification techniques whose advantage over conventional methods is the rapidity by which the test results can be obtained (Bailey, 1998).

Susceptibility to physical and chemical agents

In general, PT salmonellae are susceptible to destruction by heat with few thermo-resistant exceptions like *S. senftenberg* 775W (Schnepf and Barbeau, 1989). The USDA

and the US Food and Drug Administration both recommend cooking poultry to an internal temperature of 74°C (165 F) measured with a food thermometer (FDA, 2011; USDA, 2011a). Chemical treatments have been shown to reduce but not eliminate *Salmonella* contamination on broiler carcasses (Nassar, 1997). Chlorine has been reported to reduce the incidence of *Salmonella* contamination on broiler carcasses when applied in immersion chill tanks (Morrison and Fleet, 1985). Similarly, trisodium phosphate has been reported to reduce *Salmonella* contamination (Kim, 1994). Other chemical disinfectants with similar effects are acetic acid and lactic acid (Gast, 2003; USDA, 2010a, b).

Steps in broiler processing

Broiler processing is a series of operations involved in converting the live bird into a product that is ready-to-cook either as a whole bird or as choice cuts (Brant et al., 1982). This process begins at the farm and ends at the processing plant. The live birds are caught on farm by a live haul crew, placed in coops and transported from the farm to the processing plant. Upon arrival at the processing plant, the birds are unloaded from the coops from the trucks mechanically, and are individually placed by hand on shackles that run on a conveyor. The conveyor carries the birds through stunning, slaughtering, bleeding, scalding, defeathering, evisceration, chilling, grading, packing and shipping (Brant et al., 1982).

Stunning and bleeding

Stunning is the process of rendering the birds unconscious (Weiss, 1971). The process of stunning is essential to the humane harvesting of the birds (Sams, 2001). It

facilitates proper bleeding, and bleeding is the key to making sure that the birds are dead before passing through the scalder which is required under USDA regulations 9 CFR 381.65 b (Sams, 2001; USDA, 2006). Electrical stunning is considered most effective and economical in comparison with chemical stunning and mechanical stunning (Sams, 2001; USDA, 2006). Proper exsanguination prevents the entry of live birds into the scalder, where they would breathe in the scald water if alive, which would be an animal welfare issue. Additionally, improperly bled birds result in bright red discoloration of extremities and body surfaces of the carcass leading to carcass condemnation (Brant et al., 1982; USDA, 2006).

Scalding

Scalding is the procedure in broiler processing wherein the broiler carcasses are immersed in hot water. The scalding process facilitates defeathering because the high temperature denatures the proteins that hold the feathers in place (Sams, 2001). In the U.S., the standard scalding technique is immersion scalding which involves submerging the broiler carcasses in agitated hot water baths of 50-60° C for 2 to 2.5 minutes (Dickens et al., 1999). Another accepted method for scalding is the steam-spraying method which involves the use of hot water sprays (USDA, 2006).

Hard and soft scald

A "hard" or "soft" scald depends on the combination of temperature and length of time of scalding. In soft scald, the broiler carcass is exposed to hot water at 53°C (128°F) for 2 minutes (Sams, 2001). With this type of scald, the feathers are loosened without much damage to the cornified layer of the skin (stratum corneum). This waxy layer with

yellow color denotes good health in birds in some cultures and thus is important for export considerations (Sams, 2001). On the other hand, hard scald is when the carcasses are exposed to 62-64°C (145-148°F) for 45 seconds (Sams, 2001). With the hard scald, the waxy yellowish layer mentioned above is actually removed because of the higher temperatures but is said to result in better defeathering. This removal of the waxy layer is preferred when the end product will be coated and fried (domestic consumption) because the batter or coating is said to adhere better to the skin without the said layer (Sams, 2001).

Scalding can have its advantages and disadvantages. The advantage is bacterial reduction, but the disadvantage is cross-contamination of carcasses (Buhr et al., 2005a). Scalding removes some of the microorganisms found on the surface (feathers, skin) and feet of the carcasses: these are either killed by the hot water, or washed away in the overflow. However some microorganisms may survive and cause re-contamination of the source carcass or cross-contamination of the other carcasses in the tank (Bryan and Doyle, 1995). Other studies (Buhr, 2006) have shown that the use of multiple tanks, counter current flow, and pre-scald accessories such as brushes, helped reduce bacterial cross-contamination during scalding.

Defeathering

Defeathering is the process of feather removal. This stage in the processing has been shown to be a major control point because of the magnitude of the bacterial contamination that occurs during defeathering (Bryan and Doyle, 1995; Lillard, 1990). It has been shown that *Salmonella* on the bird's feathers and skin are able to contaminate the rubber fingers on the defeathering equipment, and these in turn can transfer the

contamination to subsequent carcasses (Bryan et al., 1968). Interestingly, studies on the effect of featherless broilers on *Salmonella* contamination resulted in no significant reduction of *Salmonella* contamination with the use of featherless strains of broilers when compared to regular broilers (Buhr et al., 2005b).

Evisceration

Evisceration is the systematic removal of the internal organs ("viscera") and trimming of processing defects from the broiler carcasses through a series of mechanized procedures done in preparation to chilling (Sams, 2001; USDA, 2006). The techniques and the sequence of events may differ from plant to plant but in general, evisceration involves opening the body cavity, and mechanically extracting the viscera (gastro-intestinal tract, reproductive tract, heart and lungs), removing the neck, the feet, the oil gland, and attachments to the vent (Sams, 2001; USDA, 2006).

Chilling

Chilling is the final step in the processing of broiler carcasses in the poultry processing plant (Sams, 2001). The primary objective of chilling is to reduce microbial growth to a level that will maximize both food safety as well as the product's shelf-life (Sams, 2001). The chilling operation directly and strongly impacts the safety and quality of the product (Tsai et al., 1992). Compliance to HACCP requirements is monitored at the end of the chilling stage by checking the prevalence of *Salmonella* in broiler carcasses as they exit the chill tank. This further highlights the importance of this stage because of the impact on the ability of the processing plants to meet regulatory standards of food safety.

In the U.S., the commercial poultry processing plants use a method known as immersion chilling, or water chilling or hydro-cooling while the European counterparts commonly use air-chilling (Sams, 2001). Chilling is carried out to reduce the temperature as well as the microbial load of the broiler carcasses (Bryan and Doyle, 1995; Kemp et al., 2000; Sams, 2001). Hydro-cooling involves subjecting the carcasses to an inside-outside bird wash (after evisceration) and then chilling them in tanks for 60 minutes or more at 4°C (39°F) (Sams, 2001). Usually, multiple stage tanks are used and the broiler carcasses are removed from the shackles and slowly pushed through the water mechanically. The tanks are filled with circulating water that has chlorine concentrations of up to 50 parts per million (ppm) (Kemp et al., 2000; USDA, 2010a). In 2010, the use of chlorine as an antimicrobial agent in poultry processing of products being exported to Russia was banned (Flynn, 2010). A few poultry companies use air chilling but the majority still use water chilling with chlorine for non-Russian export (Shire, 2010). Companies may use water chilling together with alternative antimicrobial agents if these are in the list of USDA-approved substances (USDA, 2012). Studies have shown that both air chilling and water chilling were effective in reducing microbial load, particularly *Salmonella*, but there was no significant difference between the two types of chilling methods (Huezo et al., 2007; Zhang et al., 2011).

Research has demonstrated that continuous immersion chilling significantly reduces microbial counts on the carcasses (Brant et al., 1982; Izat et al., 1989; Tsai et al., 1992) but not without the challenge of cross-contamination (Lillard, 1990).

Factor interactions affecting *Salmonella* contamination of broiler carcasses in the chill tank

Temperature and *Salmonella* growth

The growth of *Salmonella* is inhibited at or below 40˚F or 4°C (Bailey et al., 2000; Brant et al., 1982). The chilling process reduces the temperature of the chicken carcasses and delays or prevents the growth of bacteria (1998; Mead, 1989). The effect of different refrigeration and freezer temperatures on the microbiological profile of chicken carcasses was also reported by Bailey et al. (2000) who studied processed broiler chickens which were held at 4, 0, -4, -12 and -18 degrees Celsius (40, 32, 26, 10 and 0 degrees Fahrenheit). They found that *Salmonella* counts on salmonellae-positive carcasses did not change at any of the above storage temperatures (Bailey et al., 2000).

The role of pH

The definition of pH is the "negative logarithm of the hydrogen ion concentration in a solution" (Blood, 2007). In other words, it is the measure of the acidity or alkalinity of a substance (Blood, 2007). The pH of dressed chicken is 6.5-6.7 when the measurement is made on the breast meat (Young et al., 1991). The pH of chicken flesh, like that of other animal carcasses, decreases during rigor mortis due to the lactic acid accumulation in the meat and the final pH is normally reached shortly after slaughter (Bate-Smith and Bendall, 1949; de Fremery and Pool, 1960; Dodge and Peters, 1960).

The pH affects *Salmonella* growth which is best at pH 7.0 but can grow in a pH range from 4.0 to 9.0 (Gast, 2003). Sampathkumar et al (2003) found that high pH environment during trisodium phosphate (TSP) treatment destroys *Salmonella enterica* serovar *enteritidis*. Teo (1996) found that high temperature and high pH had a

synergistic effect of destroying *Salmonella enteritidis* and *Escherichia coli*. On the other hand, *Salmonella typhimurium* has been reported to develop acid tolerance response (ATR) in the presence of acidic pH from 4.5-5.8 (Foster, 1991). Citric acid and acetic acid, two of the most common organic acids used as antimicrobials in the poultry processing industry, have also been found to cause high ATR in *Salmonella* grown in meat extract (Alvarez-Ordonez, 2009).

Temperature and pH

Temperature affects pH since heating affects the dissociation of ions (White, 1999). Changing the temperature of water also changes its pH. For example, at 0°C (32°F), water has a pH of 7.5. At 25°C, water has a pH of 7.0 and at 50°C, water has a pH of 6.6 (White, 1999). Thus, in this case, it can be seen that the effect of increasing the temperature is a decrease in the pH.

Chlorine

Chlorine is a disinfectant commonly used in water systems. The use of chlorine in immersion chillers has been found to reduce *Salmonella* levels on broiler carcasses (Magwood et al., 1967; Mead, 1989; Morrison and Fleet, 1985).

Chlorine's bactericidal activity is affected by pH as well as temperature. Hypochlorous acid (HOCl) is the antimicrobial form of chlorine. The dissociation of hypochlorous acid (HOCl) into hydronium ion (H^+) and hypochlorite ion (OCl^-)is highly dependent on pH (White, 1999). At pH 4-6, 15°C and at atmospheric pressure, HOCl predominates with a concentration of 98-99%. At 20°C, pH 6.5, the concentration of HOCl is 92.28%. At pH 7.0, 20°C, the concentration of HOCl is 79.10 %. At pH 7.5, the

concentration of HOCl is 54.84% and at pH 8.0, OCl^- predominates with 72.54% concentration. At pH 8.5, OCl^- concentration is 89.31 % and at pH 9.0, OCl^- concentration is 96.35%.

Total chlorine

Total chlorine is defined as the sum of the free chlorine and the bound chlorine (chlorine that is bound to organic and inorganic matter) (White, 1999). Bound chlorine is inactive and unable to exert any antimicrobial action.

Chlorine hydrolysis

In The Handbook of Chlorination, White gives a detailed description of the chemistry of chlorination (White, 1999). If chlorine gas is dissolved in water, it results in hydrolysis of chlorine:

$$Cl_2 + H_2O \rightarrow HOCl + H + OCl^- \qquad 1.1$$

(White, 1999)

This reaction occurs rapidly and complete hydrolysis takes place within a few tenths of a second at 18 °C and within a few seconds at 0°C (White, 1999). The percentage of hypochlorous acid at freezing temperature (0°F or 32°C) is highest at pH 5.0 (99.85%) and gradually decreases to 0.01% at pH 11.7 (White, 1999). Table 1.1 shows the percent hypochlorous acid at freezing temperature at pH levels 5.0 to 7.0. excerpted from the table in "Handbook of Chlorination and Alternative Disinfectants" by (White, 1999).

The calculations that gave rise to this table were based on the equation:

$$\text{Percent HOCL} = \frac{100 * HOCl}{[(HOCL) + (Cl2) + (OCl-) + (Cl)]} \quad 1.2$$

(White, 1999)

Hypochlorous acid

Hypochlorous acid (HOCl) is a weak acid and thus it undergoes a partial dissociation.

$$\text{HOCl} \leftrightarrow \text{HOCl} + H^+ + OCl^- \quad 1.3$$

(White, 1999)

The ionization constant, K_i changes with temperature. The values of K_i have been calculated from the acid dissociation constant *pKa* (White, 1999):

$$pKa = \frac{300000}{T} - 10.0686 + 0.0253T \quad 1.4$$

(White, 1999)

Where T = 273 + degrees Centigrade.

Table 1.1 Percentage of hypochlorous acid at freezing temperature

pH	Percent HOCl at 0°C
5.0	99.85
5.5	99.53
6.0	98.53
6.1	98.16
6.2	97.69
6.3	97.11
6.4	96.39
6.5	95.5
6.6	94.4
6.7	93.05
6.8	91.41
6.9	89.42
7.0	87.04

Note: At pH 5.0 to 7.0
Adapted from White (1999)

Organic matter

Organic matter reduces the activity of chlorine (Mead and Thomas, 1973; Tsai et al., 1992). Organic material load in the chill tank affects the survival of bacteria. Organic matter contains organic nitrogen from proteins and amino acids. When organic nitrogen is present in the water, it reacts with the hypochlorous acid to form organic chloramines or organochloramine, which is non-germicidal (White, 1999). Organochloramines include monochloramine (NH_2Cl), dichloramine ($NHCl_2$) or Nitrogen Trichloride (NCl_3).

Turbidity

The current standard definition of turbidity according to the ASTM International (formerly known as the American Society for Testing and Material), is that it is "an expression of the optical properties of a sample that causes light rays to be scattered and absorbed rather than transmitted in straight lines through a sample" (Ziegler, 2002). In more simple terms, turbidity is a measure of the clarity or cloudiness of water. The turbidity of water is due to the presence of suspended particles (Sarai, 2002; Ziegler, 2002). Examples of suspended and dissolved matter are soil, clay, plankton, organic acids, dyes and matter including sewage, blood fat, minute organisms, and other microscopic organisms, (Sarai, 2002; Ziegler, 2002). High turbidity indicates a large amount of suspended particles in the water. These particles block the light and absorb additional heat from light.

Turbidity of the poultry chill tank water has been investigated (Mead and Thomas, 1973; Tsai et al., 1992; Yang et al., 2002). The amount of suspended organic matter was measured using turbidity (nephelometric readings) and found to be increasing

with an increasing number of carcasses passing through the chill tank and with a decreasing percentage of free available chlorine present (Mead and Thomas, 1973). The disinfecting action of chlorine was found to be negatively affected by dissolved organic and inorganic matter, which reduces the availability of chlorine to act against microorganisms (Tsai et al., 1992).

Turbidity and chlorine

In another study, it was demonstrated that increasing the age (in hours) of the chilled water reduced the free available chlorine levels and thus, decreased the effectiveness of chlorine in reducing *Salmonella* contamination (Yang et al., 2002). This may be due to the accumulation of organic matter in the chilled water, which happens over a period of time. This was also reported by Mead and Thomas (1973) in their study of the factors affecting the chilling of eviscerated poultry. They observed that as the number of carcasses that entered the chillers increased, the free available chlorine decreased, and the turbidity readings on the chiller water increased. Chlorine sanitizes the wash water and maintains a low microbiological count in the water. However, the main limitation of chlorine as a bactericide is that it can react with organic matter (becomes bound) which causes it to lose its effectiveness (Mead and Thomas, 1973; Russell, 2005; Tsai et al., 1992).

The use of chlorine in immersion chillers has been found to reduce *Salmonella* levels on broiler carcasses (Lillard, 1980; Magwood et al., 1967; Mead et al., 1999). Water samples were found to be significantly better microbiologically when treated with chlorine (Cl_2) 34 ppm or 5 ppm chlorine dioxide (ClO_2), than when treated with 20 ppm Cl_2 or 3 ppm ClO_2 (Lillard, 1980). The FSIS requires that the level of free available

chlorine in chill tank water is no more than 50 ppm, and no more than 5 ppm in the red (re-used) water (Sanders and Blackshear, 1971; USDA, 2003, 2012)

Water flow rate

Early beginnings of regulations regarding continuous chilling required fresh water at an input rate of 0.5 gallons (1.9 liters) per bird (USDA, 1972). The rate of water flow was found to have a significant effect on the level of turbidity in the chiller water (Mead and Thomas, 1973). Wesley (1977) found that reducing the rate of water flow from 0.5 gallons to 0.25 gallons (0.95 liter) did not affect the quality of the broiler carcass and would be more economical. Thomson et al (1979) found that reducing the fresh water input to 0.95 liter per bird to the supply water in simulated commercial chilling did not significantly affect the survival of marker salmonellae organisms inoculated into the carcasses. However, they attributed a decrease in available chlorine in the chilling water to the organic matter buildup from the carcasses (Thomson et al., 1979). On the other hand, Mead and Thomas (1973) demonstrated that varying the flow rate had a significant effect on the levels of turbidity and residual free chlorine. The microbial load has been shown to increase if the rate of addition of fresh water is insufficient (Bailey et al., 1987; Mead, 1989). USDA (2010a), in its compliance guide, recommends a high flow rate of at least one gallon per bird and a counter-current direction for the flow.

Cross-contamination in the chill tank

One of the challenges of poultry processing is cross-contamination in the chiller by *Campylobacter jejuni* or *Salmonella typhimurium* which occurs when one contaminated broiler carcass can transfer its contamination to others that were previously

free of contamination (Yang et al., 2002). The problem of cross-contamination in the chill tank was also investigated by the FSIS (James et al., 1992a; James et al., 1992b; Lillard, 1990). In the study done by the FSIS in 1987 in Puerto Rico using carcass rinse samples, it was found that chilling significantly decreased bacterial contamination on broiler carcasses but significantly increased the *Salmonella* prevalence due to cross-contamination in the chillers (James et al., 1992b). This finding was supported by a study done in two other processing plants wherein they found that the incidence of *Salmonella* spp. on broiler carcasses increased from 10-12.5% (pre-chill) to 27.5-37.5% (post-chill) (Lillard, 1990). Evidence was found by Sarlin et al. (1998) to further support that the chill tank was the most likely area where cross-contamination can occur.

Cross-contamination during chilling depends on certain conditions, namely the use of chlorine, the total residual chlorine or available chlorine concentration and the chemical composition of the chiller water (Yang et al., 2002). This is because chlorine reacts with inorganic and organic matter as well as with the micro-organisms. The incidence of *Salmonella* spp. increased significantly after chilling in non-chlorinated chill water, but remained almost the same after chilling in chlorinated water with 25 ppm chlorine (James et al., 1992a). Chlorination of chill water at concentrations lower than 30 ppm total residual chlorine did not prevent bacterial cross-contamination, while total residual chlorine higher than 30 ppm greatly reduced post-chill bacterial incidences (Mead et al., 1994). In a study by Yang et al. (2001), it was found that chlorination of chill water at 10 to 50 ppm of total chlorine was effective for reducing bacterial level in the chill water, but was not effective for reducing the bacteria attached to chicken skins.

Their results also showed that an increasing age of chill water significantly reduced the bactericidal activity of the chlorine.

Other antimicrobial agents

Trisodium phosphate (TSP) was used for years in the industry for automated reprocessing with the approval of the regulatory agencies, however, TSP has some very important limitations (Bourassa et al., 2004). Among the limitations are: first, it is used at a high concentration of ten percent, second, TSP residues on the broiler carcasses cause the chiller water pH to increase up to 10.5, resulting in the inefficient use of chlorine (Bourassa et al., 2004; Russell, 2005). The ideal pH for chlorine to form the desired hypochlorous acid, which is very effective against *Salmonella*, is 6.0 to 7.0. As mentioned previously, pH higher than 7.0 favors the formation of the hypochlorite ion, which has very low disinfecting action.

Cetylpyridinium chloride is a quaternary ammonium compound. It is also approved by the FDA for use in poultry processing for ready to cook poultry products. The pH of the compound is almost neutral; it is water soluble and volatile. It is effective against a broad range of pathogens including *Salmonella* (USDA, 2010a).

Other antimicrobial compounds approved by the FDA include organic acids such as lactic acid, acetic acid, citric acid, and oxalic acid. Organic acids have some organoleptic effects on the meat, thus their use may be limited in the processing industry (USDA, 2010a).

Models and simulation modeling

Models are constructs of reality (Forrester, 1961). A model is a representation of anything that is in the real world. It can be a physical representation such as a model car. It can be a mathematical model, which is a representation of a phenomenon in numerical terms, such as an equation for the growth of bacteria in a petri dish or the trajectory of a cannon ball. Simulation is a process applied to a model. A simulation is a series of solutions to an equation (Hannon and Ruth, 1997). Webster's dictionary defines a simulation as the representation of or prediction of the results of a process or a series of processes by means of computers (Morehead and Morehead, 1995).

Predictive modeling

For the past two decades, predictive modeling has been widely developed in the field of food safety (Rosso et al., 1995; van Impe et al., 1992). This field combines knowledge of bacterial growth responses over a range of conditions and the power of mathematical modeling to predict the growth of the organisms in a given set of conditions. Predictive modeling in food safety risk assessments has simulated bacterial growth as a function of temperature, pH and water activity. Kinetic models for spoilage and pathogenic organisms have been developed and incorporated into publicly available applications software such as the Pathogen Modeling Program of the USDA ARS, the Food MicroModel (McClure et al., 1994), the Seafood Spoilage Predictor (Dalgaard et al., 2002), and the Food Spoilage Predictor (Neumeyer, 1994).

Cross-Contamination Probability Model

A predictive model was adapted for the probability of *Campylobacter jejuni* and *S. typhimurium* cross-contamination during chilling by Yang et al. (2002). In the experimental study, inoculated and non-inoculated chicken drumsticks were mixed in a chiller simulator and the post-chill contamination (positive/negative) was determined for each drumstick.

They adapted the probability model:

$$P = \frac{1}{1+e^{-y}} \qquad 1.5$$

where P is the probability of an individual chicken drumstick being contaminated after chilling and *y* is a linear function of treatment factors, pre-chill incidence (%), total chlorine level in chill water (ppm), and the age of the chill water (h).

Although useful, these advances in predictive modeling are still found to be wanting. The classic predictive model shows the growth of bacteria in the face of changes in various parameters (Rosso et al., 1995; van Impe et al., 1992). When compared to the classic predictive models, the kinetic models go further with predicting the growth with changes in parameters over time (Dalgaard et al., 2002). Yang et al.'s (2002) cross contamination probability model goes even further by considering the changes in the parameters, time, and the interactions between the parameters . Absent from all the above models is the feedback loop (which is a representation of a feedback mechanism), which is one of the most important characteristic found in complex biological systems (Forrester, 1971; Hannon and Ruth, 1997). The ability to model the

feedback loops, and other complex non-linear relationships in systems, is the distinct advantage in using system dynamic models over other predictive model types.

Types of models

Hannon and Ruth (1997) described three general types of models, namely static, comparative static and dynamic. Static models represent a snapshot of reality at a specific point of time. Comparative static models as can be deduced from its name compare several static models (Hannon and Ruth, 1997). A good example would be a time series of maps of a disease epidemic. Each of the maps is a static model, but the aggregation of the maps, presented in a time-series, is a comparative static model. The third type of model, the dynamic model, represents and captures changes over time (Hannon and Ruth, 1997). This type enables the modeling of complex and diverse systems using a powerful and user friendly software (Hannon and Ruth, 1997). The advantage of dynamic modeling is the ability to model biological systems (Hannon and Ruth, 1997). This type of model is the focus of the research reported in this dissertation.

Risk assessment

Risk assessment has been conducted on *Salmonella* contamination in whole chickens from retail to the table and proper cooking (Oscar, 2004). In the same study, the role of the consumer in proper handling and cooking of the chicken was evaluated as a risk reduction measure. Grijspeerdt et al. (2005) used individual-based modeling to model growth and migration of *Salmonella enteritidis* in hens' eggs. Parsons et al. (2005) used Bayesian Network, Markov Chain Monte Carlo (MCMC) and Monte Carlo simulation modeling approaches for quantitative risk assessment for *Salmonella* in

poultry meat from breeder farm to the chilled carcass. However, these applications were found to be limited by Parsons et al. (2005). They found that Bayesian Network (BN) is sensitive to changes and requires all the variables to be discrete, which introduces errors if continuous variables have to be converted to discrete variables. They also found that the MCMC approach does not require discrete variables while retaining some of the properties of the BN model, such as the ability to draw inferences from evidence. The Monte Carlo simulation modeling approach was found to be more flexible but more complicated. Another shortcoming of the Monte Carlo simulation modeling approach used by Parsons et al (2005) was that they did not have data about the organisms in every stage of the model. Modeling techniques have been used for modeling *Salmonella* growth and risks in the poultry production continuum, but not system dynamics simulation modeling.

System dynamics modeling

System dynamics (SD) is that branch of modeling that recognizes and takes a broad view or the holistic approach when any form of organized system is being studied (Martin et al., 1987). The holistic approach is based on the concept that parts of a system are interrelated and linked by some form of feedback mechanism. (Martin et al., 1987; Morley, 1972).

The system dynamics approach was initially proposed by Jay W. Forrester, an engineer, who was involved in the U.S. government's project of building the prototype of computers (Forrester, 1961). System dynamics offers an alternative perspective on management and heuristics. System dynamics modeling looks at the interrelationships between the factors involved in a system and allows for the inclusion of feedback loops

which are important components of complex systems in nature ((Forrester, 1971; Hannon and Ruth, 1997).

Models have various advantages and functions. Models, being representations of real systems, allow for the demonstration of the significant parts of the system. They allow understanding of the mechanisms at work. In using models instead of real systems, alternative operations or scenarios could be attempted without incurring the real-life losses that could occur if the alterations were done on the real system (Hannon and Ruth, 1997).

The system dynamics simulation approach relies on understanding complex interrelationships existing between different elements within a system (Elshorbaggy et al., 2005). This is achieved by developing a model that can simulate, and quantify the behavior of the system. System dynamics simulation modeling has been applied to various industries including the medical and health sciences, as well as in studies of the environment (Elshorbaggy et al., 2005). However, it has not been applied to processing of poultry. There are no existing system dynamics models of microbial contamination that can be modified or validated or otherwise used in this research. In particular, there are no existing models of the system of *Salmonella* contamination of broiler carcasses in the chill tank in a poultry processing plant. In the same manner, this current study aims to obtain a better understanding of the system of the *Salmonella* contamination of the chicken carcasses in the chill tank of a poultry processing plant, and the interrelationships between the factors that are involved.

STELLA modeling program

Structure Thinking, Exponential Learning Laboratory with Animation (STELLA®) is a user-friendly graphical programming software that uses simple icons (Richmond and Peterson, 2000). The STELLA® classification of variables is simplified and the resulting icons associated with them can be modified to capture the various parts that influence a system's behavior.

The software, STELLA®, is one of the popular commercially available simulation modeling programs (Hannon and Ruth, 1997). Icons such as stocks, flows, converters, and connectors are used in STELLA®. It is a modeling language and a program for graphic modeling and animation which is fairly accessible because it does not require knowledge of computer programming (Hannon and Ruth, 1997; Richmond and Peterson, 2000). Traditionally, modeling required knowledge of programming such as Fortran, C+ or other languages (Hannon and Ruth, 1997).

According to Elshorbaggy et al (2005), a system dynamics model using STELLA® must be simple but dynamic, rely on available data but is expandable with additional data. It must have a feedback, and it is able to simulate shocks that may happen to the system.

Validating system dynamics models

Model validation

Several definitions of validation have been recorded. According to Coyle (1977), it has to deal with the practicality of the model. Giannanasi et al. (2001) defined validation as the process of assessing the suitability of the model based on its correctness.

On the other hand, for Balci (1998), model validation deals with testing that the model remains true to the objectives of both the model and the simulation.

Validation can be considered as one of the most important parts of the modeling process since it determines the sensibleness of the model that has been developed. Solberg (1992) said that the validity of a model determines its power Model validation has been found to be more problematic for system dynamics models including STELLA (Martis, 2006; Mohapatra, 1987).

Validation techniques

Several model validation techniques have been developed (Barlas, 1989, 1996; Forrester and Senge, 1980; Khazanchi, 1996; Martis, 2006).

Model validation technique by Forester and Senge (1980)

Under this technique by Forester and Senge (1980) the model is validated by its objective, structure, behavior and policy implications. The model is subjected to tests for the model structure and behavior which consist of questions about the structure and behavior and how they relate to the real system being modeled. For example, model structure is tested for suitability by checking the compatibility of the model structure with the real system, and its ability to withstand shocks applied to it. Model structure consistency is validated by the face validity test – how similar is the model structure to the system being modeled and by questions referring to consistency of the parameters used in the model and in reality. Model structure usefulness is tested by the appropriateness of the model to the audience for the study. Model behavior is similarly validated according to suitability, consistency and usefulness. For example, for

suitability of model behavior, sensitivity analysis may be done to check if the model is sensitive to variations.

Model validation scheme by Khazanchi (1996)

This validation technique is more applicable to qualitative models and consists of eight questions about the model, namely : "is the model plausible, reasonable, feasible, effective, pragmatic, empirical, predictive, and certifiable ?" (Khazanchi, 1996).

Other techniques

Face validity

Simply put, this technique seeks the opinion of experts if the system model behavior is reasonable (Forrester, 1961; Law and Kelton, 1982; Naylor and Finger, 1967). After a model has been formulated and run, there is a need to validate it. To validate is to answer the question "how representative of reality is the model?" (Naylor and Finger, 1967).

A three step approach to model validation by Naylor and Finger (1967)

This technique has been modified by Law and Kelton (1982). The steps are 1) begin with a model with high face validity, 2) perform empirical testing, and 3) determine how representative the simulation output data are.

A model with high face validity is one that is accepted as a reasonable representation of the system by experts in the field (Law and Kelton, 1982). The second step, empirical testing may be carried out by comparing observed data with literature data (Khazanchi, 1996; Law and Kelton, 1982). The third step is testing the "representative-ness" of the simulation data. Actual observed data are compared with model predicted

data, and the predicted data should be very close to the reality if the model is valid (Law and Kelton, 1982).

Statement of the problem

Salmonella contamination of broiler carcasses is a threat to the health of individual consumers, to food safety, and to the livelihood of millions of families who are employed by the poultry industry. Although much has been learned about the microbiology of *Salmonella*, there are still gaps in knowledge of its ecology, particularly the relationships between the parameters involved with *Salmonella* contamination in the chill tank stage of the processing. System dynamics modeling has not been previously used in risk modeling for the poultry processing industry. In addition, no studies in actual chill tanks in commercial processing plants have been done that comprehensively included determination of changes in the six parameters, namely 1) pH, 2) temperature, 3) chlorine concentration and 4) turbidity level in the chill tank water, 5) pre-chill and 6) post-chill *Salmonella* contamination status of the broiler carcass, and the relationships among these parameters over time. Developing a system dynamics simulation model for *Salmonella* contamination at the chilling stage of poultry processing will provide a powerful tool for assessing the risk of *Salmonella* contamination in the chiller as well as for implementing intervention strategies. The objective of this research is to 1) find the best sampling method to use when testing whole carcass rinse samples; 2) develop a system dynamics simulation model of *Salmonella* contamination of broiler carcasses in the chill tank of the poultry processing plant based on literature; 3) find the relationships among factors/parameters in the chill tank of a poultry processing plant by collecting data on pH, temperature, turbidity, and level of chlorine using a specially designed apparatus;

and 4) develop a system dynamics model of *Salmonella* contamination of broiler carcasses in the chill tank of a poultry processing plant, that is able to predict the probability of a single broiler carcass exiting the chill tank as positive using actual pre-chill and post-chill prevalence data from whole carcass rinse samples and recorded measurements of turbidity, pH, and chlorine levels in a poultry processing plant in Mississippi.

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CHAPTER II

AGREEMENT OF THREE WHOLE CARCASS RINSE SAMPLING METHODS (ADJACENT PAIR, CONSECUTIVE, AND SPLIT CARCASS) ON THE DETECTION OF *SALMONELLA* CONTAMINATION

Abstract

Whole carcass rinse is the most common method used to determine *Salmonella* prevalence in broiler carcasses. However, there is a need to find the most sensitive whole carcass rinse sampling method that allows repeated assessment of the *Salmonella* status of a broiler carcass as it proceeds through processing.

Three whole carcass rinse sampling methods, namely adjacent pair method, consecutive method, and split-carcass method, were evaluated to determine which method was the most sensitive and most accurate. The adjacent pair method is where two broiler carcasses found side by side on the processing line are sampled and their *Salmonella* status is compared with each other. The consecutive pair method is where a broiler carcass is subjected to consecutive whole carcass rinses and the samples compared as to *Salmonella* status. The split carcass method is where the broiler carcass is split into two halves and each half is subjected to a whole carcass rinse and the *Salmonella* status of the samples is compared. Five trials were conducted in poultry processing plants in the southeastern US. Whole carcass rinse samples were collected from broiler carcasses at the pre-chill stage, and tested in the laboratory for the presence or absence of

Salmonella. The results were analyzed and compared by Kappa agreement and McNemar's Test.

The consecutive pair method had the highest but also the lowest agreement, showing inconsistent results. Also, in one trial, the consecutive pair method showed significant difference in prevalence rates between consecutive rinses. The disadvantages of the split carcass method were that it was more labor- and time- intensive and the product was damaged. The adjacent pair sampling method was found to be the most preferable whole carcass rinse method to use when studying *Salmonella* status or prevalence. The adjacent pair sampling method showed moderate agreement consistently (Kappa =0.46, 0.54, 0.46 for Trials 3, 4, and 5 respectively, $p<0.0001$).

Keywords: *Salmonella*, agreement, Kappa, broiler carcass, sampling.

Introduction

Assessing the efficacy of intervention measures applied during processing to control *Salmonella* relies on accurately measuring the *Salmonella* status of the broiler carcasses being treated. The whole carcass rinse (WCR) procedure has been widely used in studies of *Salmonella* prevalence in broiler carcasses (Cason et al., 1997; Cox et al., 1983; Izat et al., 1991; James et al., 1992a; James et al., 1992b; Jetton et al., 1992; Jimenez et al., 2002; Lillard, 1989). One example of these studies is Cason et al. (1997) who reported a comparison between the prevalence of *Salmonella* and *Campylobacter* in pre- and post-immersion chill tank (pre-chill and post-chill) carcasses using WCRs in their study on the relationship between aerobic bacteria, *Salmonella* and *Campylobacter*. In another study using WCR's, Jimenez et al. (2002) compared the pre-chill and post-

chill prevalence of *Salmonella* on broiler carcasses and the presence or absence of visible fecal matter.

The statistical power of studies measuring intervention efficacy in poultry systems could be increased if the same carcass were measured before and after the treatment was applied rather than relying on changes in prevalence in groups of broilers. The use of paired carcass halves (one half for control and the other for treatment) was suggested by Izat et al. (1990), as a possible means of reducing variability in trials designed to test the effectiveness of various chemicals in reducing or eliminating *Salmonella* within processing waters. In their studies which looked at most probable number (MPN) estimates of *Salmonella* in the carcass halves, they found that the variation between the two halves of a split carcass was equal and significantly less than the variation among individual whole carcasses (Izat et al., 1990). Jetton et al. (1992) used paired broiler carcass halves in their study comparing recovery of *Salmonella* from chilled broiler carcasses using different rinse media (distilled water and physiological saline) and enumeration methods namely, MPN and dulcitol-novobiocin agar (DNB). There were no significant differences in the bacteria recovered from carcass halves rinsed with either physiological saline or distilled water in the study by Jetton et al. (1992). However. no paired carcass halves were rinsed in the same media. Cason and Berrang (2002) tested the findings of Izat et al. (1990) by comparing the MPN of bacteria on paired broiler carcass halves, and reported these to be valid on aerobic bacteria, *Campylobacter*, *E. coli* and other coliforms. They concluded that paired carcass halves had the advantage of reduced variability in numbers of bacteria over counts on different carcasses.

Carcass to carcass variability in *Salmonella* numbers in WCR studies has been reported by several authors (Cason and Berrang, 2002; McNab et al., 1993; Renwick et al., 1993). In a study of variability in broiler carcass bacterial load, a series of five repeat rinses were conducted on one bird from each of 96 study lots (McNab et al., 1993). They found positive association between the bacterial count of the first rinse and the subsequent rinses and a declining trend in the counts in the subsequent rinses (McNab et al., 1993).

In another study, Renwick et al. (1993) investigated the variability and determinants of carcass bacterial load from WCR's of roaster chickens. The samples were collected from the evisceration line of a commercial poultry abattoir over a 5 month period and identified as to the lot and supplier. Carcass-to-carcass variability accounted for 73.2% of the total variability in bacterial loads of pre-chill carcasses. The remainder of the variability in bacterial load was attributed to between-lots-within-supplier (14.2%), and between-supplier (12.6%) , respectively.

The comparison of consecutive rinses from a broiler carcass has been studied with conflicting results (Izat et al., 1991; Lillard, 1989). Lillard (1989) found that *Salmonella* was not always recovered from the initial carcass rinse but was recovered from subsequent carcass rinses of the same broiler carcass. On the other hand, in a study done to determine the accuracy of a single WCR in estimating the MPN of *Salmonella*, no significant difference was found in four consecutive carcass rinses of the same broiler carcass that had been inoculated with *Salmonella typhimurium* (Izat et al., 1991).

Measuring agreement by the Kappa statistic

The Kappa (κ) coefficient of agreement was initially proposed by Cohen (1960) as a measure of agreement between two raters using nominal scales. Kappa is the proportion of agreement after chance agreement is removed (Cohen, 1960). The value of Kappa has an upper limit of 1.00 signifying perfect agreement while the lower limit falls between zero and -1.00. A useful scale for the interpretation of Kappa was proposed by Landis and Koch (1977) to classify the strength of agreement namely, Kappa less than 0 equals "poor" agreement, 0.00 – 0.20 as "slight", 0.21 – 0.40 as "fair", 0.41-0.60 as "moderate", 0.61-0.80 as "substantial" and 0.81-1.00 as "almost perfect".

Some problems have been reported about the use of Kappa statistic – specifically about the effect of prevalence (Cicchetti and Feinstein, 1990; Feinstein and Cicchetti, 1990; Sierra and Cardenas, 2007). One of the non-chance-corrected indices of agreement discussed by Cicchetti and Feinstein (1990) is the Odds-Ratio (OR). Odds ratios greater than 1 represent higher agreement, and a value of 1 represents purely chance agreement (Cicchetti and Feinstein, 1990). However, the limitation of the odds ratio as an index of agreement is that it is useless in cases of very low prevalence or finding any zeros in the 2X2 contingency table (Cicchetti and Feinstein, 1990).

Objectives of the study

The goal of this research was to determine a sampling method that could be used to best assess the *Salmonella* contamination status of individual broiler carcasses before and after an intervention/treatment on the processing line (i.e. chilling). Simply rinsing the same carcass before and after the treatment seems to be the most straight forward approach but raises concerns that the initial rinsing procedure might impact the results of

the second rinse. An alternative approach is to rinse one carcass prior to the treatment and a carcass that was immediately adjacent to the first carcass on the processing chain after the treatment. This method assumes that the adjacent carcass would share the *Salmonella* status of the first carcass. A third approach would be to split a carcass into two halves, rinse each half separately and compare the *Salmonella* status. The objective of this study was to determine which of these three methods would provide the best assessment of *Salmonella* status by measuring the agreement between paired samples when an intervention was applied in between the samples.

Materials and methods

Five trials were conducted to evaluate the three different sampling methods. The trials were conducted at commercial poultry processing plants in the southeastern U.S. Two trials were conducted to test the agreement for the split carcass rinse method and three trials were conducted to determine the agreement for the adjacent and re-rinse carcass rinse methods.

Split carcass rinse method

For the split carcass rinse method, the broiler carcasses were removed from the processing line at the same site as for the adjacent and consecutive samples using fresh latex gloves, placed in individual sterile plastic bags, placed in a cooler and immediately taken to a place at the plant off the processing floor where the carcasses were split into two halves each. A total of 150 carcasses were sampled for each trial. Each carcass was aseptically divided in the midline using a new pair of autoclaved scissors and each half (A and B) was placed in an individual sterile plastic bag with 100 ml of Butterfield's

solution and shaken for one minute according to established procedure (Cox et al., 1983; Izat et al., 1990). The split carcass rinse samples were collected in individual sterile Nalgene® (NalgeNunc International, Rochester, NY 14625, USA) containers and transported on wet ice to the laboratory for *Salmonella* isolation.

Consecutive vs. adjacent WCR

In order to evaluate the consecutive pair (re-rinse) and adjacent pair (adjacent carcass rinse) sampling methods, broiler carcasses were sampled by pairs, removed from the processing line at the pre-chill stage before they entered the chill tank. In each of the three trials 150 pairs of broiler carcasses were sampled using WCR. A pair of broilers was comprised of two adjacent carcasses on the processing line. Using fresh latex gloves for each carcass, a pair of carcasses was removed from the processing line after the inside outside spray wash but before the final rinse cabinet. Each broiler carcass was then placed inside separate sterile plastic bags with 100 ml of Butterfield's solution, and shaken for one minute as previously described (Cox et al., 1983). The first of the pair of carcasses was returned to the line after the rinse sample was collected. Its rinse sample was labeled with its pair number and an "adjacent" designation. Following its first rinse, the second of a pair of carcasses was placed in a second sterile plastic bag with Butterfield's solution and subjected to a second WCR. The first and second rinses of this carcass were labeled by pair number and designated "Rinse" and "Re-rinse", respectively. After the second rinse sample was collected, the second broiler carcass was returned to the processing line. The rinse samples were collected in individual sterile Nalgene® (NalgeNunc International, Rochester, NY 14625, USA) containers and transported on wet ice to the laboratory for *Salmonella* isolation.

Salmonella isolation

For all trials, the combination of tetrathionate (TT) (Remel Inc., Lenexa, KS 66215) broth and Rappaport-Vassiliadis (RV) broth selective enrichment method previously described (Rybolt et al., 2004) was used to isolate *Salmonella*. Briefly, the broiler carcass rinses were brought up to 1x Buffered Peptone Water (BPW) with addition of 10x BPW. The sample was mixed and then incubated for 24 hours at 42ºC. One ml of the rinse sample was transferred to a tube containing 9 ml of TT broth. The tube was vortexed and incubated at 42 C for 18-24 hours. From the tube, 0.1ml was transferred to a tube containing 9.9 ml of RV broth. The tube was vortexed and then incubated at 42 C for 18-24 hours. A loopful of RV broth was streaked on an XLT4 agar plate and incubated at 37 C for 18-24 hours. *Salmonella*-like colonies were confirmed biochemically using Triple Sugar Iron (TSI) agar (Difco Laboratories, Detroit, MI 48232) and Lysine Iron Agar (LIA) (Difco Laboratories, Detroit, MI 48232), and serologically using Anti-*Salmonella* Poly A-I and Vi serum (Difco Laboratories, Detroit, MI 48232).

Sample size

One hundred fifty pairs of broiler carcasses or split-carcass halves were collected for each of the carcass rinse method trials. Power analysis using Cantor's sample size equation (Cantor, 1996) estimated that for each trial the sample size of 150 pairs would allow the discrimination between Kappa values for two methods of 0.4 and 0.6 assuming an alpha level of 0.05, power of 0.80, and prevalence estimates of 0.50 by either method.

Statistical analysis

Assessing agreement and differences in prevalence for the different methods was performed using the FREQ procedure of SAS for Windows version 9.3 (SAS Institute, Inc. Cary, North Carolina). The Tables/Agree statement generated the Kappa coefficient of agreement between adjacent and rinse samples for the adjacent pair method; between rinse and re-rinse samples for the consecutive pair method; as well as between half A and half B for the split carcass method within each of the appropriate trials. An overall Kappa for each method was also determined by controlling for trial. The Kappa coefficients were interpreted according to the categories suggested by Landis and Koch (1977). McNemar's Test was used to determine if the prevalence of *Salmonella* was different between adjacent and rinse samples for the adjacent pair method, between rinse and re-rinse samples for the consecutive pair method, as well as between half A and half B for the split carcass method within each of the appropriate trials. An analysis of variance using PROC ANOVA (SAS for Windows v9.3) was conducted to determine if the mean Kappa values for each method and trial were different. A significance level of 0.05 was used for all analyses.

Results and discussion

Split carcass method Trial 1

The prevalence of *Salmonella* in the half A was 81.3% and 78.7% in half B (Table 2.1). McNemar's Test was used to compare the prevalence between the paired samples and no significant difference between the prevalence rates found in half A and half B was found (p=0.37). This finding however does not necessarily mean that the *Salmonella* status of individual pairs of samples were the same. To measure the degree

of agreement between the half A and half B samples on their *Salmonella* status, Kappa coefficients were determined. The Kappa agreement for Split Carcass Method in Trial 1 was 0.58 or moderate agreement under the Landis and Koch method of interpretation. The results showed that both halves of the split carcasses agreed (concordant pairs) that 20 (13.3%) were negative and 110 (73.3%) were positive for *Salmonella* contamination. There were 20 pairs (13.3%) of split carcass halves that did not agree (discordant pairs).

Split carcass method Trial 2

In Trial 2 of the Split Carcass method, the *Salmonella* prevalence was 63.3% on half A and 70.7% on half B. McNemar's Test showed no significant difference between prevalence rates of *Salmonella* between half A and half B in this trial (p=0.071). The percent positive samples for half A and half B can be seen in Table 2.1.The Kappa coefficient of agreement was 0.45 for the split carcass method in Trial 2. This is interpreted as moderate agreement. Both carcass halves A and B agreed (concordant pairs) that 31 (20.67%) were negative and 82 (54.67 %) were positive. There were 37 discordant pairs (24.67%).

Comparing Trial 1 and Trial 2, of the split carcass method, Trial 1 had the higher Kappa coefficient; however, both trials were considered to have moderate agreement. Results of Trials 1and 2 are found in Tables 2.1 and 2.2. The overall Kappa for the split carcass method controlling for trial was 0.51, which is moderate agreement also and the test for equal Kappa showed that there were no significant differences between the Kappa coefficients in each trial (p= 0.221) from analysis controlling for trial.

Consecutive rinses and adjacent broiler carcasses Trial 3

The percent positive samples for adjacent, rinse and re-rinse in Trial 3 can be found in Table 2.1. The prevalence of *Salmonella* was 24.7% in the adjacent, 24.7% in the rinse, and 20.0 % in the re-rinse broiler carcasses. Using McNemar's Test to compare the paired samples, there were no significant differences between the prevalence of *Salmonella* in the adjacent carcasses (p=1.0), nor between the consecutive rinses of the same broiler carcass (p=0.11). To measure the degree of agreement between samples, Kappa coefficients were determined. The Kappa coefficient in this case, measures the degree to which adjacent broiler carcasses and consecutive carcass rinse samples agree on the presence or absence of *Salmonella* contamination. The comparison between adjacent broiler carcasses showed that both broiler carcasses agreed (concordant pairs) that 22 (14.7%) were positive and 98 (65.3%) were negative for *Salmonella* contamination. However, there were 30 (20.0%) adjacent broiler carcass pairs that did not agree (discordant pairs). The Kappa value for the adjacent broiler carcasses was 0.46 (p< 0.0001). This is considered moderate agreement following Landis and Koch (1977). It is possible that there was only moderate agreement due to greater variation in *Salmonella* contamination status between adjacent broiler carcasses which is contrary to the assumption that adjacent carcasses on the processing line have similar *Salmonella* contamination status.

Table 2.1 Percent positive samples and McNemar's Test P values for adjacent, rinse and re-rinse samples

Trial	Adjacent	Rinse	Re-Rinse	Split Carcass		McNemar's Test P value		
				A	B	A vs R*	R vs RR	A vs B
1				81.3	78.7			0.371
2				63.3	70.7			0.071
3	24.7	24.7	20.0			1.000	0.108	
4	68.7	69.3	66.0			0.853	0.398	
5	17.3	22.7	12.0			0.117	0.002	

*A vs R denotes Adjacent vs Rinse; R vs RR denotes Rinse vs Re-Rinse; A vs B denotes carcass half A vs carcass half B

Table 2.2 Kappa agreement values among adjacent pairs, consecutive pairs, and split carcass rinses

Trial	Comparison	Kappa	p-value	Interpretation**
1	Split Carcass Rinse	0.58	<0.0001	Moderate
2	Split Carcass Rinse	0.45	<0.0001	Moderate
3	Adjacent Pairs	0.46	<0.0001	Moderate
	Consecutive Pairs	0.64	<0.0001	Substantial
4	Adjacent Pairs	0.55	<0.0001	Moderate
	Consecutive Pairs	0.47	<0.0001	Moderate
5	Adjacent Pairs	0.46	<0.0001	Moderate
	Consecutive Pairs	0.41	<0.0001	Moderate

Note: ** According to Landis and Koch (1977)

It has been hypothesized that *Salmonella* cross-contamination of adjacent carcasses can occur during the inside-outside spray wash (Jimenez et al., 2002). However, their study was based on an increase in *Salmonella* prevalence from carcasses visibly contaminated with feces at the shower stage compared to the prevalence from carcasses with visible fecal contamination at the evisceration stage, rather than a comparison of actual adjacent carcasses. In a study of broiler carcasses processed with an inside-outside bird washer, no cross-contamination of adjacent carcasses was found when uncontaminated broiler carcasses were placed adjacent to contaminated carcasses

during washing (Smith et al., 2005). The absence of cross-contamination was attributed to the use of pilot size 30.5 cm (12 in.) shackle center separation distance as opposed to the standard used by industry which is 15.3 cm (6 in.). In the current study, pairs of adjacent carcasses were identified and the *Salmonella* contamination statuses were compared from their individual WCR samples, assuming standard industry shackle separation distance.

In this research, the comparison between the consecutive WCR samples yielded 131 concordant pairs (24 pairs *Salmonella* positive and 107 pairs *Salmonella* negative). The Kappa value for the agreement between consecutive rinses was 0.64 ($p<0.0001$). This agreement was higher compared to that found in adjacent pairs rinse method and this degree of agreement is interpreted as "substantial" based on the scale by Landis and Koch (1977). Compared to the agreement between adjacent broiler carcasses, this relatively better agreement may be attributed to lower variation since consecutive WCR samples from the same broiler carcass were being compared. This is similar to the findings of Izat et al. (1991) who found no significant differences in MPN of *Salmonella* between four consecutive rinses of the same broiler carcass that had been contaminated with *Salmonella* spiked samples.

It was interesting to note that even though the *Salmonella* prevalence rates in the adjacent birds were very similar, there was only moderate agreement. This shows that adjacent broiler carcasses did not have uniform *Salmonella* contamination status at the pre-chill stage. This finding was important because it suggested that the rate of cross-contamination of broiler carcasses as they were side by side on the processing line was low which agrees with the findings of Smith et al. (2005).

Consecutive rinses and adjacent broiler carcasses Trial 4

The percent positive samples for adjacent, rinse, and re-rinse in Trial 4 can be found in Table 2.1. The prevalence of *Salmonella* was 68.7% in the adjacent, 69.3% in the rinse, and 66.0% in the re-rinse broiler carcasses. Using McNemar's Test to compare the paired samples, there were no significant differences between the prevalence of *Salmonella* in the adjacent carcasses (p>0.853), nor between the consecutive rinses of the same broiler carcass (p>0.398). The comparison between adjacent broiler carcasses showed that both broiler carcasses agreed (concordant pairs) that 89 (59.3%) were positive and 32 (21.3%) were negative for *Salmonella* contamination. However, there were 29 (19.3%) adjacent broiler carcass pairs that did not agree (discordant pairs). The Kappa value for the adjacent broiler carcasses was 0.55(p < 0.0001) (Table 2.2). This is considered moderate agreement following Landis and Koch (1977).

In Trial 4, the comparison between the consecutive WCR samples yielded 115 concordant pairs (84 pairs *Salmonella* positive and 37 pairs *Salmonella* negative). The Kappa value for the agreement between consecutive rinses was 0.47 (p<0.0001). This agreement is lower compared to that found in adjacent pairs rinse method and is also interpreted as "moderate" based on the scale by Landis and Koch (1977). Compared to the agreement between adjacent broiler carcasses, this slightly lower agreement may be attributed to variation between multiple rinses of the same carcass. This type of variation between consecutive rinses was also reported by Lillard (1989).

Consecutive rinses and adjacent broiler carcasses Trial 5

The percent positive samples for adjacent, rinse and re-rinse in Trial 5 can be found in Table 2.1. The prevalence of *Salmonella* was 17.3% in the adjacent, 22.7% in

the rinse, and 12% in the re-rinse broiler carcasses. Using McNemar's Test to compare the paired samples, there was no significant difference between the prevalence of *Salmonella* in the adjacent carcasses (p=0.117), but there was a significant difference between the prevalence of *Salmonella* in the consecutive rinses of the same broiler carcass (p=0.002). The comparison between adjacent broiler carcasses showed that both broiler carcasses agreed (concordant pairs) that 17 (11.3%) were positive and 107 (71.3%) were negative for *Salmonella* contamination. However, there were 28 (17.3%) adjacent broiler carcass pairs that did not agree (discordant pairs). In this trial, the Kappa value for the adjacent broiler carcasses was 0.46 ($p < 0.0001$) (Table 2.2). This is considered moderate agreement following Landis and Koch (1977).

In Trial 5, the comparison between the consecutive WCR samples yielded 124 concordant pairs (13 pairs *Salmonella* positive and 111 pairs *Salmonella* negative). In Trial 5, the Kappa value for the agreement between consecutive rinses was 0.41 (p<0.0001). This agreement is less than that found in adjacent pairs rinse method and is also "moderate" based on the scale by Landis and Koch (1977).

It can be noted that the average prevalence of *Salmonella* was observed to be highest in Trial 4 and lowest in Trial 5. In both Trials 3 and 4, there were no statistically significant differences in the prevalence of *Salmonella* for adjacent, rinse and re-rinse samples. In Trial 5, however, there was a statistically significant decrease in the *Salmonella* prevalence between the first and second rinse. This may be an effect of the second rinse in removing some of the contamination. The greatest agreement among the three trials was found in the consecutive rinses in Trial 3. However, the highest prevalence of *Salmonella* was found in Trial 4. The overall Kappa when controlling for

trial was 0.50 for the adjacent pairs method and 0.52 for the consecutive pairs method. Tests for equal Kappa coefficients showed that there were no significant differences between the Kappa coefficients across the trials for adjacent pairs method (p=0.664) and for the consecutive pairs method (p=0.116).

Overall Comparisons

Considering the results of analysis, the highest and lowest Kappa agreement was observed among consecutive rinses of the same carcass. The highest or substantial agreement (0.64) was observed in consecutive rinse method in Trial 3, while the least and moderate agreement (0.41) was observed also in consecutive rinse method in Trial 5. On the other hand, the adjacent rinses, showed a consistently moderate Kappa agreement, without any significant differences in the prevalence between adjacent rinses. Although admittedly underpowered, the analysis of variance did not detect significant differences in the mean Kappa values among the sampling methods (p=0.958).

Since the consecutive rinses of the same carcass showed inconsistent agreement among three trials (ranging from moderate to substantial) and showed a significant difference in the prevalence between the consecutive rinses in at least one trial, it appears that the consecutive rinse is not the best method to use when checking for *Salmonella* contamination using the carcass rinse method. The significant difference indicates that rinsing the same carcass twice may be washing off the *Salmonella* resulting in the second carcass rinse to be negative. These results show that the concern regarding the impact of the two rinses on the *Salmonella* contamination status of the sampling unit is a valid concern. From the results of this study, the more appropriate method to use would be

adjacent carcasses – rinsing one of the pair prior to the intervention and then rinsing the second of the pair after the intervention.

The split carcass method also showed moderate agreement, however, this method was found to be more time and labor intensive compared to the standard carcass rinse used. Due to these limitations, the split carcass would not be the method of choice of researchers conducting a major in-plant research project involving numerous carcasses. Based on the results of this study, the Adjacent Pairs sampling method is the recommended and preferred method when using whole carcass rinse sampling in *Salmonella* status or prevalence studies. This sampling method showed moderate agreement consistently through the three trials, and there was no significant difference in *Salmonella* prevalence between the adjacent pairs. Using this sampling method in *Salmonella* status or prevalence studies would increase the accuracy of the results from such studies.

Conclusions

In summary, it is important to note that similar prevalence estimates between two populations do not necessarily mean agreement. Kappa analysis and McNemar's test are useful statistical methods to compare the agreement between sampling methods for qualitative data and for comparing matched pairs in a nominal 2x2 table. The results of this study demonstrated that there was highest agreement in *Salmonella* status between consecutive rinses of the same broiler carcass. However, the lowest agreement in the same parameter was also found between consecutive rinses of the same broiler carcass. The overall Kappa value for Trials 3-5 was 0.50 for adjacent pairs and 0.52 for consecutive rinses, virtually the same although the Kappa values for the adjacent pairs

was more consistent across the three trials. There was also a significant difference in one trial in the prevalence of *Salmonella* between the consecutive rinses of the same broiler carcass, indicating a decontamination effect of the carcass rinse process. The overall Kappa value for Trials 1 and 2 was 0.51 for the split carcass method, comparable to the other two methods. Thus, the results may be interpreted to mean that the adjacent pair method may be the better sampling method to use since it gives more consistent agreement than the consecutive rinse method, and also there was no significant difference in the prevalence found between adjacent carcasses. This information, collected under commercial processing plant conditions, can be used to design studies to evaluate intervention strategies or treatments for the control of *Salmonella* in poultry processing plants. The study results suggest that application of a treatment to one of a pair of adjacent carcasses and comparing the outcome to the untreated second of the pair is better than re-rinsing the same carcass when accessing the presence or absence of *Salmonella* following treatment.

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CHAPTER III

LITERATURE-BASED SYSTEM DYNAMICS MODEL OF *SALMONELLA* CONTAMINATION OF BROILER CARCASSES IN THE CHILL TANK OF A POULTRY PROCESSING PLANT

Abstract

Salmonella contamination of broiler carcasses continues to be a challenge for the poultry processing industry. During processing, the risk of contamination of broiler carcasses at the level of the chill tank is complex, dynamic and involves several factors. These factors include the status of contamination of the broiler carcasses as they enter the tank, dwell time of the carcasses in the chill tank, as well as characteristics of the water in the chill tank, i.e., the temperature, pH, level of chlorine, flow rate and turbidity (a reflection of the level of organic matter). In this research, a system dynamics model was developed using Structure Thinking, Exponential Learning Laboratory with Animation (STELLA®) software and available literature to simulate *Salmonella* contamination of broiler carcasses in the chill tank and to predict cross-contamination. The model stochastically generates the contamination status of each broiler carcass (positive or negative) as it enters the tank based on a pre-determined pre-chill *Salmonella* prevalence. If positive, the carcass may either remain positive or become negative (decontaminated) as it exits the chill tank. If negative pre-chill, the carcass may either become positive (cross-contaminated) or remain negative (no cross-contamination). The model

stochastically generates the status of cross-contamination and decontamination based on the pre-chill *Salmonella* prevalence, chlorine level, and turbidity of the chiller water. The model allows the initial chlorine, pH, pre-chill prevalence, and flow rate of the water to be set prior to running the simulation. Development of this STELLA model promises to provide a powerful tool for use by both regulatory and industry personnel in conducting risk assessment of simulated *Salmonella* contamination at the level of the immersion chill tank in broiler processing.

Keywords: system dynamics, model, *Salmonella*, broiler carcass

Introduction

Salmonella as a public health problem

Managing risks related to product caused illnesses is a major focus of the modern 21st century food production industry, with pathogens identified as the main cause of foodborne disease. The food animal industry considers consumer safety threats of the highest order of importance and has worked to address this issue. Poultry ranks as the highest amount of annual consumption of meat products estimated at nearly 100 pounds per capita (Council, 2013). *Salmonella* contamination of broiler carcasses continues to be a challenge for the poultry processing industry.

It has been reported that *Salmonella* causes an estimated 1.2 million illnesses in the United States (U.S.) annually (Scallan et al., 2011). *Salmonella* infections, which cause the largest numbers of illnesses, hospitalization and deaths of any pathogen under surveillance in the Foodborne Diseases Active Surveillance Network (FOODNET), have not declined over the past 15 years and actually increased since 2006-2008 (CDC, 2011).

In 2011, the incidence was nearly three times the 2010 national health objective target of 6.28 incidence rate per 100,000 population (CDC, 2011).

Processed broiler carcasses are frequently contaminated with *Salmonella* and other micro-organisms (Panisello et al., 2000). *Salmonella* contamination of broiler carcasses poses a public health hazard to the consumers (CDC, 2011). Prevention of *Salmonella* contamination is one of the priorities established by the USDA in its goal of enhancing protection and safety of the nation's agriculture and food supply (USDA, 2005).

Importance of the immersion chill tank

The immersion chill tank (chill tank) is the final stage of the evisceration section of poultry processing (Sams, 2001; Yang et al., 2002). Many studies have been done on the prevalence of *Salmonella* contamination before and after chilling, with various kinds of antimicrobial compound treatments and using different sampling methods (Cox et al., 1983; Izat et al., 1991; James et al., 1992a; Lillard, 1989). The Food Safety Inspection Service (FSIS) monitors regulatory compliance relative to the *Salmonella* performance standard of a broiler processing facility after the final intervention, which is usually as the carcasses exit the chill tank. (Federal Register, 1996; USDA, 2010a). While it is known that reduction of the carcass temperature to 4.4 °C (40 °F) for control of bacteria, including pathogens, is the objective of the chill tank process, the chiller has been shown to be the most likely source of cross-contamination for the broiler carcasses (Sarlin et al., 1998).

Factors affecting *Salmonella* contamination of broiler carcasses

There have been numerous factors studied relative to their impact on the *Salmonella* contamination of broiler carcasses. Among the factors studied are the status of contamination of the broiler carcasses as they enter the tank, dwell time of the carcasses in the chill tank, as well as characteristics of the water in the chill tank, i.e., the temperature, pH, level of chlorine, flow rate and turbidity. Various aspects of some of the factors are discussed immediately below.

The role of pH

Salmonella can grow within the pH range of 4.0 to 9.0, but has optimum growth at pH 7.0 (Gast, 2003). A high pH environment can destroy *Salmonella enterica* serovar *enteritidis* such as would be encountered during trisodium phosphate (TSP) treatment of broiler carcasses (Sampathkumar et al., 2003). Teo (1996) found that high temperature and high pH had a synergistic effect on destroying *Salmonella enteritidis*. On the other hand, *Salmonella typhimurium* has been reported to develop acid tolerance response (ATR) in the presence of acidic pH from 4.5-5.8 (Foster, 1991). Citric acid and acetic acid, two of the most common organic acids used as antimicrobials in the food processing industry, have been found to cause high ATR in *Salmonella* grown in meat extract (Alvarez-Ordonez, 2009).

Cross-contamination in the chill tank

Use of chilling to control *Salmonella* in broilers can be complicated due to cross contamination between the carcasses. In a study done by the Food Safety Inspection Services (FSIS) in 1987, researchers found that chilling significantly reduced the overall

bacterial numbers but significantly increased the *Salmonella* prevalence due to cross-contamination in the chillers (James et al., 1992b). This finding was further supported by a study done in two other processing plants where it was found that the incidence of *Salmonella* spp. increased from 10-12.5% (pre-chill) to 27.5-37.5% (post-chill) (Lillard, 1990). Results of the work of Sarlin et al. (1998) demonstrated that the chill tank was a likely component of broiler processing where *Salmonella* could spread between carcasses due to cross-contamination. A cross-contamination probability model was developed using experimentally inoculated chicken drumsticks in a simulator chill tank (Yang et al., 2002).

Chlorine and the chill tank water environment

Chlorine is the main chemical used worldwide to control pathogens in water to make it potable (White, 1999). The impact of chlorination of water on *Salmonella* prevalence in broiler carcasses has also been investigated (Lillard, 1980; Sanders and Blackshear, 1971; Tsai et al., 1992). Cross-contamination during chilling depends on certain conditions, namely: the use of chlorine, the total residual chlorine concentration and the chemical composition of the chiller water. This is because chlorine reacts with inorganic and organic matter as well as with other micro-organisms (Yang et al., 2002). The incidence of *Salmonella* spp. increased significantly after chilling in non-chlorinated chill water, but remained almost the same after chilling in chlorinated water with 25 ppm chlorine (James et al., 1992a). Chlorination of chill water at concentrations lower than 30 ppm total residual chlorine did not prevent bacterial cross-contamination, while total residual chlorine higher than 30 ppm greatly reduced post-chill bacterial incidences (Mead et al., 1994). In a study by Yang et al. (2001), it was found that chlorination of

chill water at 10 to 50 ppm of total chlorine was effective for reducing bacterial levels in the chill water, but was not effective for reducing the bacteria attached to chicken skins. They attributed this reduced bactericidal activity of the chlorine to increased organic matter load.

System Dynamics Modeling

System dynamics (SD) is the branch of modeling which recognizes and takes a broad view or the holistic approach when any form of organized system is being studied (Martin et al., 1987). This approach considers it important to study a system as a whole including the complex interconnections like feedback, rather than studying each part separately (Martin et al., 1987; Morley, 1972). System dynamics modeling looks at the interrelationships between the factors involved within a system (Elshorbaggy et al., 2005). In the same manner, this current research seeks to obtain a better understanding of the *Salmonella* contamination of the chicken carcasses in the chill tank by looking at it as a system, and the complex relationships between the factors that are involved.

System dynamics simulation modeling using the STELLA program has been applied to various industries including medical and health sciences, as well as in studies of the environment (Elshorbaggy et al., 2005). The STELLA program is a highly intuitive and user-friendly program which has the advantage of not requiring prior training in computer programming (Richmond and Peterson, 2000). However, to date, neither SD nor STELLA has been applied to processing of poultry. There are no existing SD models of microbial contamination that can be modified or validated or otherwise used in this research. In particular, there are no existing models of the system of

Salmonella contamination of broiler carcasses in the chill tank in a poultry processing plant. This is the first endeavor to look at the *Salmonella* contamination as a system.

Salmonella research has been conducted for more than a hundred years and yet, its public health importance has not diminished. There is a need to apply the SD approach to *Salmonella* contamination in order to better understand the system and provide a tool for testing interventions. System dynamics has been applied to other fields in biology but not in the poultry processing industry (Elshorbaggy et al., 2005; Hannon and Ruth, 1997). System dynamics as a world view looks at the relationships between factors in a process and views these factors as part of a system (Forrester, 1971).

Salmonella contamination in the chill tank can be considered as a system that is influenced by several factors namely, the processing line speed in evisceration, the prevalence of *Salmonella* on the broiler carcasses entering the chill tank, chill water characteristics i.e., temperature, pH, chlorine level, turbidity, and flow rate. In this study, a system dynamics model of *Salmonella* contamination of broiler carcasses in the chill tank of a processing plant was developed using available literature.

The objectives of this research are 1) to develop a system dynamics simulation model of the *Salmonella* contamination of broiler carcasses at the level of the chill tank in the poultry processing plant utilizing existing literature, and 2) provide a unique, useful and innovative tool for testing interventions for both the regulatory sector and the poultry companies in reducing the risk of *Salmonella* contamination.

Materials and methods

Software: STELLA®

STELLA® Software version 8.1 (iseesystems™, Lebanon, NH) was utilized in this study. Structure Thinking, Exponential Learning Laboratory with Animation (STELLA®) is a user-friendly graphical programming program that uses simple icons (Richmond and Peterson, 2000). The STELLA® classification of variables is quite simple and the resulting icons associated with them are quite appropriate for capturing all the parts that influence a system's behavior.

The software, STELLA®, is a popular commercially available simulation modeling program (Hannon and Ruth, 1997). Icons representing such things as stocks, flows, converters, and connectors are used in STELLA®. It is a modeling language and a program for graphic modeling and animation which is fairly accessible because it does not require knowledge of programming (Hannon and Ruth, 1997; Richmond and Peterson, 2000). Traditionally, modeling required knowledge of programming such as Fortran, C+ or other languages (Hannon and Ruth, 1997). Furthermore, a system dynamics model using STELLA® can be simple and yet flexible, dynamic and sensitive to shocks that may be applied to the system leading to better decisions since different policies or management scenarios may be tested using the model (Elshorbaggy et al., 2005).

Statistical Analysis

Regression analysis using PROC REG, SAS for Windows version 9.3 (SAS Institute, Inc., Cary, NC) was utilized to model the relationship between turbidity (dependent variable) and the independent variables of the number of carcasses that have

gone through the chill tank and the water flow rate in the chiller. A linear regression equation was developed from data published by Mead and Thomas (1973) to relate the number of carcasses processed over time. This equation was then used to extrapolate the number of carcasses to be processed in 5 hours in a commercial processing plant. Data relating time, water flow, and turbidity from the same study was then used to develop a multiple regression equation to estimate turbidity of chiller water as a function of time and water flow rate. These readings were then combined as a graphical function with the data of Yang et al. (2002) to make age of chiller water as a function of turbidity. This step was necessary in order to use a multiple logistic regression equation predicting the contamination of a single chicken drumstick by *S. typhimurium* during processing developed by Yang et al. (2002). The explanatory variables used in the model were age of chiller water, pre-chill prevalence, and chlorine level. The regression model was used as the basis for estimating the cross-contamination probability (Y) in the STELLA® model. The occurrence of *Salmonella* on inoculated chicken drumsticks following mock chilling, as reported in Table 2 of Yang et al. (2002), was used to develop a logistic regression model predicting the occurrence of *Salmonella* as a function of chlorine and age of chiller water. PROC LOGISTIC (SAS for Windows v 9.3) was used to run the model. The logistic regression model was used to develop the logit of the decontamination probability (Z).

Results and discussion

Model development and formulation

First, the scope of the system dynamics model had to be limited such that it is a model of the *Salmonella* contamination at the level of the processing plant. The model

(Figure 3) begins with the arrival of the chickens at the processing plant (arrival). The chickens then enter the conveyor system and the speed of the line from an actual processing plant was used to represent the length of time it takes for the chickens to actually go through the stages of processing until it reached the chill tank. The point in time right before the broiler carcass enters the chill tank is termed "Pre-chill". At this point, the broiler carcasses either have *Salmonella* contamination or they do not. The Status Split conveyor was used to represent this. The pre-chill prevalence variable was connected to the Status Split conveyor to provide the pre-chill prevalence rate.

Depending on the value of the pre-chill prevalence of *Salmonella*, the birds that enter the chill tank are divided into pre-chill positive and pre-chill negative. Within the chill tank, some of the pre-chill positive broiler carcasses will become decontaminated due to the chlorine level in the chill tank, and some will stay positive as they come out of the chill tank. On the other hand, some of the broiler carcasses which were pre-chill negative could be cross-contaminated and become positive, while some could stay negative. Thus some broiler carcasses exit out of the chill tank negative for *Salmonella* contamination (post-chill negative) and some would exit the chill tank positive for *Salmonella* contamination (post-chill positive).

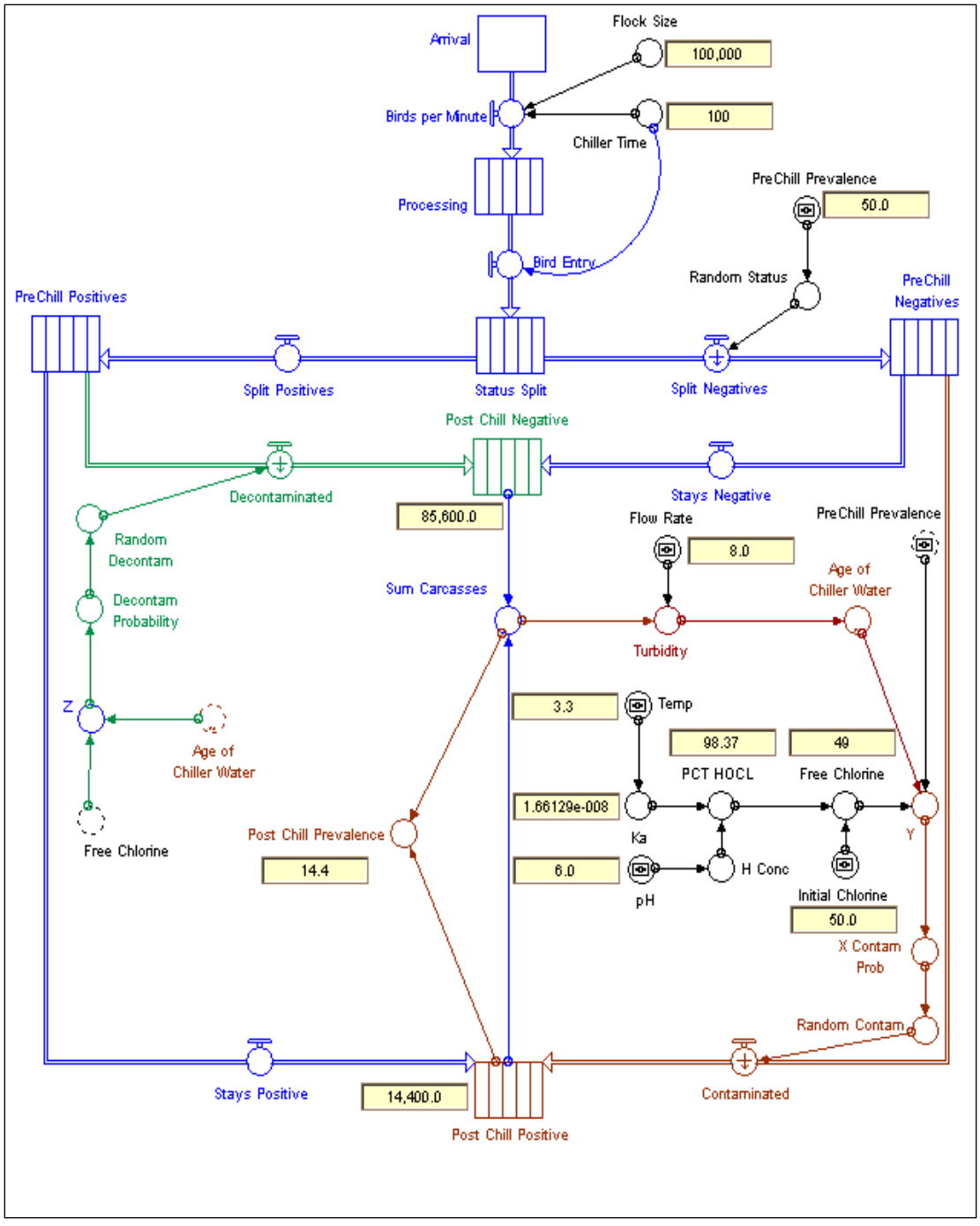

Figure 3.1 The SD model of *Salmonella* contamination of broiler carcasses in the chill tank of the poultry processing plant

Cross contamination probability model

For the pre-chill negative broiler carcasses that get cross-contaminated in the chill tank, the cross contamination probability model by Yang et al. (2002) was adapted:

$$P = \frac{1}{1+e^{-y}} \quad 3.1$$

where P is the probability of individual chicken carcass being cross-contaminated in the chiller, and exiting the chill tank positive for *Salmonella* contamination; and

where *Y* is a function of treatment factors : pre-chill prevalence, total chlorine level in chill water (ppm), and age of chiller water and is the logit of the multiple logistic regression model from Yang et al. (2002).

$$Y=-3.5099-(0.0336 \times \text{free chlorine}) + 0.0583 \times \text{pre-chill prevalence}) + 0.886 \times \text{age of chiller water} \quad 3.2$$

where free chlorine was the reported chlorine level, pre-chill prevalence was the pre-chill percent contamination, and age of chiller water was given the value of -1 when water age was 0 to 4 hours and a value of 1 when water age was 5 to 16 hours.

Decontamination probability model.

A decontamination probability model was developed from a study of inoculated chicken legs that became free of *Salmonella* following mock chilling (Yang et al., 2002).

$$P = 1-\left\{\frac{1}{[1+e^{-z}]}\right\} \quad 3.3$$

where Z = -1.3018-(0.0319*free chlorine)+(0.8767*age of chiller water); free chlorine was the reported chlorine level; and age of chiller water was given the value of -1 when water age was 0 to 4 hours and a value of 1 when water age was 5 to 16 hours.

Turbidity vs. age of chiller water

The time required to process 20,000 chickens from hanging on the shackles through the chiller was calculated as 100 birds per minute. Hence at the end of 300 minutes or 5 hours, 30,000 birds would have passed through the chiller.

The linear regression equation developed to relate the number of carcasses processed over time was:

$$Carcasses = -240.19 + 33.082\ x\ Number\ of\ Minutes \quad 3.4$$

Therefore, at 300 minutes it was estimated 9684.4 carcasses would be processed in the equipment used by Mead and Thomas (1973). This number divided into 30,000 provided the conversion factor (3.1) to transform the numbers used by Mead and Thomas to a commercial level of chicken processing. The second linear regression model using data from the Mead and Thomas (1973) study that related turbidity of chiller water to the number of carcasses processed and the flow rate used in the chiller is expressed below:

$$Turbidity = (17.72 + 0.00092\ x\ sum\ of\ carcasses) - 1.85\ x\ flow\ rate \quad 3.5$$

The graphical function tool in STELLA was used to plot turbidity versus the age of chiller water as used in the logistic regression equation developed by Yang et al. (2002) relating the occurrence of *Salmonella* to age of chiller water, chlorine levels, and pre-chill prevalence. The age of chiller water took on values of -1, 0 and 1 as the turbidity values varied from 6, 23 and 40.

pH and chlorine

The hydrolysis of chlorine occurs in the chill tank as a function of pH, temperature, the dissociation constant Ka, and the initial chlorine level. The temperature affects the dissociation constant Ka as expressed in the following equation:

$$Ka = 10\text{^} - ((3000/(Temp + 273)) - 10.0686 + (0.0253 * (Temp + 273))) \quad 3.6$$

where Temp is the temperature in degrees Celsius.

The proportion of chlorine which is in the form of hypochlorous acid is expressed by the following equation:

$$Percent\ HOCl = (1/(1 + (Ka/H\_Conc))) * 100 \quad 3.7$$

where Ka is the dissociation constant and H_Conc is the hydrogen ion concentration which is a function of pH. The free available chlorine which is used in calculating Y of the cross-contamination probability model is determined by multiplying percent HOCl by the initial chlorine level.

Contamination and decontamination

The cross-contamination probability was then used in conjunction with a random number generator function with STELLA® to assign a leak fraction value to the leakage flow regulating the number of pre-chill negative carcasses that become post-chill positives. The decontamination probability was similarly placed into a random generator that would randomly assign positive or negative to each event of a broiler that had entered the chill tank as *Salmonella*-positive. The pre-chill positives that get decontaminated (post chill negative) are added to the other post-chill negatives, i.e. those broiler carcasses that were pre-chill negative for *Salmonella* and stayed negative when

they exited the chill tank. The STELLA® program automatically subtracts the number of post chill negative broiler carcasses from the total of pre chill positive broiler carcasses then adds the post chill positives together and the post chill negatives together. Thus, the post chill positives are the sum of the pre-chill positives that stayed positive when they exited the chill tank and the pre-chill negatives that became cross-contaminated in the chill tank. On the other hand, the post-chill negatives are the sum of the pre-chill negatives that stayed negative and the pre-chill positives that became decontaminated in the chiller.

Post-chill prevalence

The sum of carcasses is the total cumulative number of broiler carcasses that entered and exited the model. The flock size which is given also has a predetermined estimated pre-chill prevalence of *Salmonella* contamination. As the broiler carcasses went through the model processing and chilling, the cross-contamination probability model and the decontamination probability model resulted in a particular number of post-chill positives. This number was then compared by the model with the sum of carcasses which is the total population of broiler carcasses that entered through the system at that point in time and thus the post-chill prevalence of *Salmonella* contamination is determined.

SD model of *Salmonella* contamination in the poultry processing plant

The SD model of the *Salmonella* contamination in the chill tank of the poultry processing plant was thus designed and developed using the STELLA program. The available knowledge of processes involved in processing and the available data from

literature were used to build this model. Whenever data were needed but not available, e,g, data about relationships among the factors, calculations were done using meta-analysis, where possible, to find the appropriate relationship. The first question to be asked was whether or not it could be done, i..e., could system dynamics modeling or systems thinking approach be used or applied in a biological situation like *Salmonella* contamination, in the poultry processing plant? The results of the study would indicate that the answer to the question would be in the affirmative. Using data from the literature, and available knowledge, a literature based SD model of *Salmonella* contamination in the chill tank was developed, and successfully run in simulations. It was able to mimic the system, as well as the processes that go into the system, the entry of the broiler carcasses into the chiller, the *Salmonella* prevalence of the carcasses, the cross-contamination probability of the carcasses in the chiller, the decontamination probability of the carcasses in the chiller, and the chlorine level, pH level, temperature, turbidity, and age of chiller water. All of these factors could be quantitatively or qualitatively entered into the model, based on available knowledge and meta-analysis of data from the literature. The SD model developed shows that indeed it is possible to apply system dynamics modeling to the field of broiler processing. It was also very interesting to be able to simulate the processing plant, particularly the *Salmonella* contamination of the carcasses, using the SD model. It is of particular interest to find that the simulations resulted in the expected interactions and outcomes as reported in the literature.

Traditional predictive modeling has allowed researchers to learn much about the linear relationships between *Salmonella* contamination or growth and the various factors

involved. However, it must be realized that there may be relationships present in the process that are complex and indirect, as opposed to simple and direct ones, and that dynamic feedback mechanism may be involved as well. The SD model is able to predict the post-chill prevalence of *Salmonella* in broiler carcasses in a flock, if the pre-chill prevalence, pH, age, and turbidity of the chiller water are known. Figure 3.2 shows the predicted post-chill *Salmonella* positive and negative broiler carcasses over a period of time. It demonstrates that over time, the number of post-chill *Salmonella* negative carcasses increase, and also the number of post-chill *Salmonella* positive carcasses increase but not at quite as high a level. The number of birds that have arrived at the processing plant is also simulated and at the end of the processing cycle time.

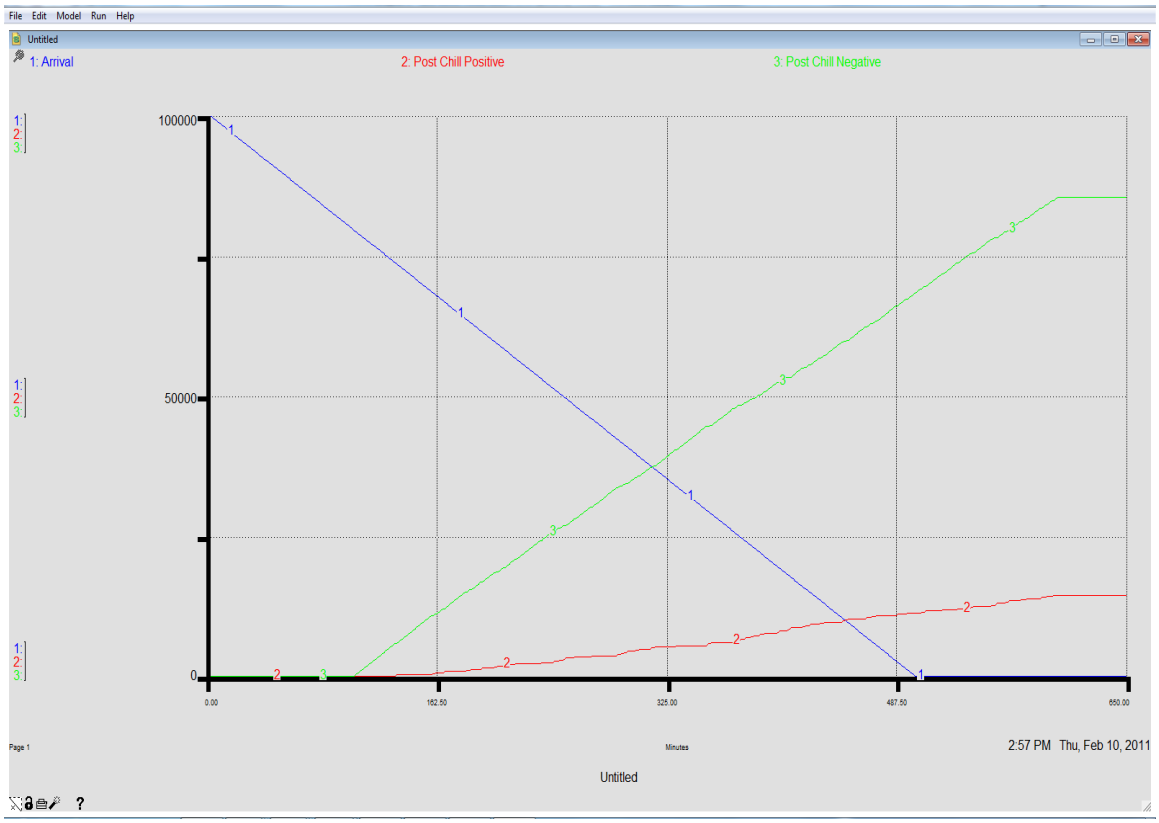

Figure 3.2 Predicted post-chill *Salmonella* positive and *Salmonella* negative broiler carcasses

The SD model is able to predict the post-chill prevalence of *Salmonella* contamination. Figure 3.3 shows the increasing post-chill positive broiler carcasses during various simulations, with a pre-chill prevalence of 50%. Figure 3.4 shows the predicted post-chill prevalence, with a pre-chill prevalence of 25%. This demonstrates the potential and power of this tool for testing interventions and scenarios in the chill tank of the processing plant.

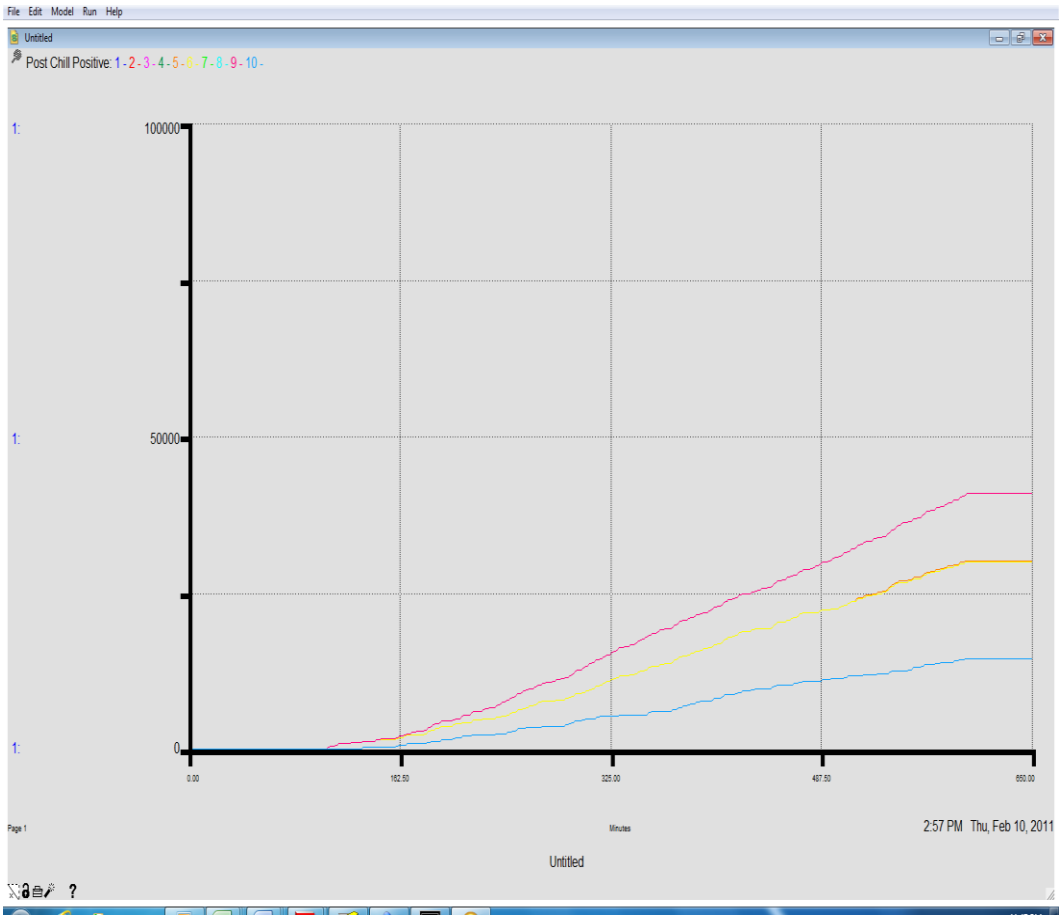

Figure 3.3 Post-chill *Salmonella* positive broiler carcasses as predicted by simulations, at pre-chill *Salmonella* prevalence of 50%

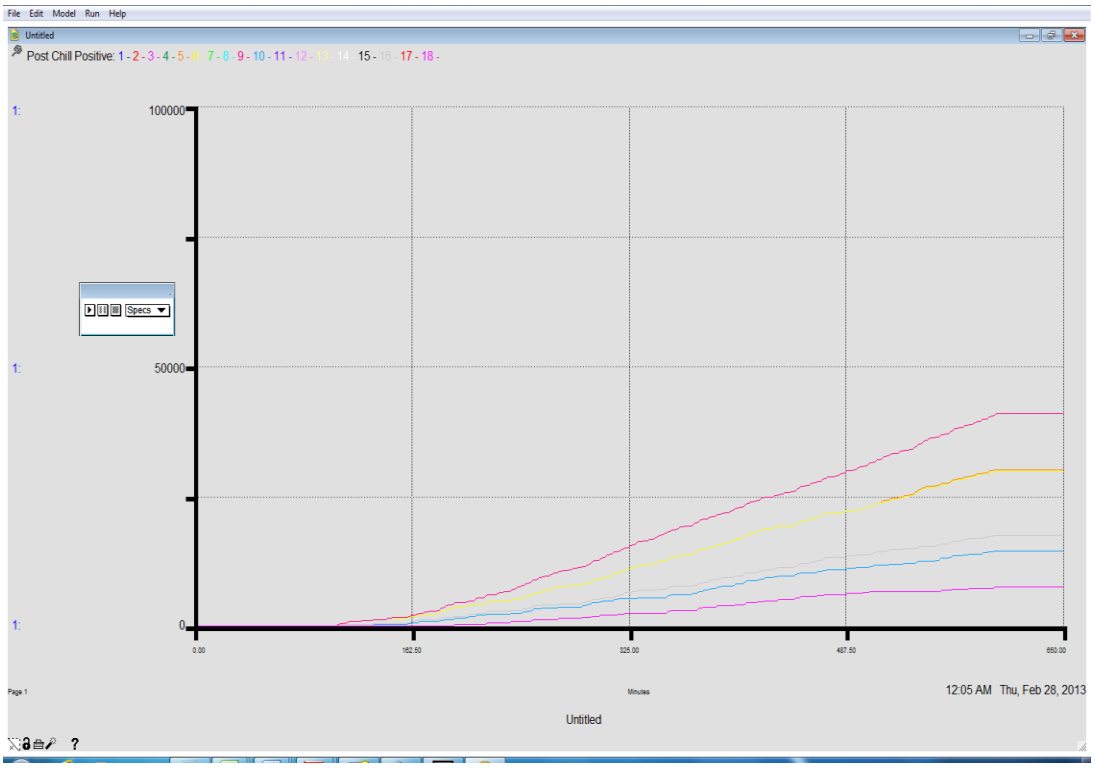

Figure 3.4 Post-chill *Salmonella* positive broiler carcasses predicted, at pre-chill *Salmonella* prevalence of 25%

Conclusions

Through this research we are able to present a system dynamics model of *Salmonella* contamination occurring in the chill tank in a broiler processing plant. After a thorough search of the literature, we believe this is the first report of the development of a simulation model for the modeling the *Salmonella* contamination system occurring in the chill tank of a broiler processing plant. This work demonstrates how a literature-based SD model was developed and shows that this type of simulation modeling is applicable for *Salmonella* contamination studies in the poultry processing industry. The model is able to represent the various processes occurring in the target system and the related processes occurring before and after the target system in the poultry processing plant.

The SD model is able to apprise the water immersion chill tank in the plant in its attributes like pH, temperature, turbidity, and age of chiller water. The model shows that the *Salmonella* contamination is a system made up of numerous factors. It is able to simulate the relationships between these factors and their ultimate effect on the outcome: cross-contamination or de-contamination in the chill tank. Thus, the STELLA program for system dynamics modeling is applicable in the field of food processing in simulating food safety issues, particularly for modeling *Salmonella* contamination. It has the advantage of allowing for qualitative data and uncertainties which still abound in the study of *Salmonella* contamination. The model developed has the potential to be a powerful tool for use by regulators as well as broiler companies in simulating and testing interventions for reducing *Salmonella* contamination in the processing plant. Simulating the levels of free chlorine, pH, turbidity, and inputting the pre-chill *Salmonella* prevalence into the model based on available information would be less costly than running actual trials. A future improvement on this model can be done by developing the model based on actual data.

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CHAPTER IV

THE RELATIONSHIPS BETWEEN VARIOUS FACTORS AFFECTING *SALMONELLA* CONTAMINATION IN THE CHILL TANK OF A POULTRY PROCESSING PLANT

Abstract

The objective of this study was to determine the relationship between factors affecting *Salmonella* contamination of broiler poultry carcasses at the chill tank stage in the poultry processing plant. The prevalence of *Salmonella* contamination in broiler carcasses before entering (pre-chill) and after exiting (post-chill) the chill tank, at varying levels of the parameters temperature, pH, chlorine, and turbidity over time were measured during seven trials conducted in one poultry processing plant in Mississippi from June to September 2010.

Temperature, pH, free chlorine (hypochlorous acid only), and turbidity levels were measured in the water immersion chill tank and recorded over time using a specially designed apparatus that was comprised of a temperature probe, a pH probe, a chlorine 0-5 ppm probe, and a turbidity probe. The apparatus was connected to a data logger which recorded the values every minute during the sampling. Mixed-effects linear regression analysis was conducted to determine the relationship between turbidity and minute, hypochlorous acid (HOCl) level and turbidity, pH and minute, pH and temperature, and temperature and minute. There was a significant relationship between turbidity and

minute ($p<0.0001$). As minute increased, turbidity increased with the model equation: $\text{Turbidity} = 11.2576 + (0.1443 \times \text{minute})$. There was a significant relationship between HOCl and turbidity ($p<0.0001$) with the model $HOCl = 1.4143 - (0.00281 \times Turbidity)$. As turbidity increased, HOCl decreased. There was also a significant relationship between the temperature and minute ($p<0.0001$) with the model: $Temperature = 6.2747 + (0.004768 \times Minute)$. The apparatus assembled was effective in measuring the variables, and could potentially be useful to the processing industry.

Keywords: Chill tank, turbidity, chlorine, pH, temperature

Introduction

The water immersion chill tank has been shown to be an area where cross-contamination with *Salmonella* can occur on the broiler carcasses (Lillard, 1990; Yang et al., 2002). The chill tank can be conceived of as a system composed of various factors- a dynamic system of *Salmonella* contamination. These factors include chlorine, temperature, turbidity, and pre-chill salmonella incidence/prevalence. Some studies have looked at the impact that changing these factors have on the *Salmonella* prevalence. The objective of this study was to investigate the relationships of the factors with one another at the level of the water immersion chill tank.

Chilling

Chilling is the final step in the processing of broiler carcasses in the poultry processing plant. The primary objective of chilling is to reduce microbial growth to a level that will maximize both food safety as well as the product's shelf-life (Sams, 2001).

The chilling operation directly and strongly impacts the safety and quality of the product (Tsai et al., 1992). Compliance with Hazard Analysis Critical Control Point (HACCP) requirements is monitored at the end of the chilling stage by checking the prevalence of *Salmonella* in broiler carcasses as they exit the chill tank (USDA, 2006). This highlights the importance of this stage because of the impact on the ability of the processing plants to meet regulatory standards on pathogen reduction, health and food safety and ultimately public health. Research has demonstrated that with the processing methods used today, continuous water immersion chilling significantly reduces microbial counts on the carcasses (Bailey et al., 2000; Brant et al., 1982; Izat et al., 1989; Tsai et al., 1992) but not without the challenge of cross-contamination (Lillard, 1990).

The regulatory agency Food Safety Inspection Services (FSIS) requires that carcass temperature on exit from the chiller shall be 2°C to 4°C (36˚ F to 40˚F) because *Salmonella* and other human enteric bacteria do not multiply at 4°C or below (Bailey et al., 2000; Brant et al., 1982). The roles pH and chlorine levels of the chiller water play in microbial reduction have been the subject of investigations.

Factors affecting *Salmonella* contamination of broiler carcasses in the chill tank

The factors affecting *Salmonella* contamination of broiler carcasses in the chill tank are temperature, pH, turbidity, and chlorine level. Chilling temperature (temperature at or below 40˚F or 4°C) inhibits *Salmonella* growth (Bailey et al., 2000; Brant et al., 1982).

A properly operating chill tank could achieve effective bacterial reduction but it is crucial to have the right pH, temperature, flow rate and direction, chlorine concentration

and concentration of organic material in order for the chlorine in the chiller to work (Stopforth et al., 2007; Tsai et al., 1992; Yang et al., 2001).

The pH affects *Salmonella* growth. *Salmonella* grows best at pH 7.0 but can grow at pH range from 4.0 to 9.0 (Gast, 2003). Sampathkumar et al. (2003) found that high pH during trisodium phosphate (TSP) treatment destroys *Salmonella enterica* serovar *enteritidis*. Teo (1996) found that high temperature and high pH had a synergistic effect of destroying *Salmonella enteritidis* and *Escherichia coli*. A low pH on the other hand stimulates the Acid Tolerance Response (ATR) in *Salmonella (*Foster*,* 1991*)*

The use of chlorine in immersion chillers has been found to reduce *Salmonella* levels on broiler carcasses (Magwood et al., 1967; Mead, 1989; Morrison and Fleet, 1985; Tsai et al., 1992). Chlorine's bactericidal activity is affected by pH. Hypochlorous acid (HOCl) is the most germicidal species of all the chlorine compounds or fractions (White, 1999). It is also known as the free available chlorine residual (Farkas et al., 1949; White, 1999). The germicidal efficiency of HOCl is due to the ease with which the HOCl can penetrate cell walls. Hypochlorous acid is structurally comparable to water. It is small in molecular size, and has no electrical charge (White, 1999).

The bactericidal action of chlorine is by oxidizing the bacterial cell. The hypochlorous acid and hypochlorite ion attack micro-organisms and bacteria in the water by penetrating the cell wall, destroying the lipids in the cell wall and oxidizing enzymes inside the cell. This abolishes enzyme action and results in bacterial destruction (Green and Stumpf, 1946; White, 1999; Wyss, 1962).

The dissociation of hypochlorous acid (HOCl) into hydrogen ion (H^+) and hypochlorite ion (OCl^-) is highly dependent on pH (Morris, 1966; White, 1999). At

lower pH, the HOCl fraction is favored, while at higher pH, the hypochlorite ion is favored. At lower temperatures, the HOCl fraction is favored and at higher temperature, the dissociation to hypochlorite ion is favored.

The concentration of chlorine in water in the form of dissolved gas (Cl_2), hypochlorous acid (HOCl), and/or hypochlorite ion (OCl^-) is referred to as free chlorine (White, 1999). In contrast to free available chlorine, free chlorine may be bound to organic matter. Any residual chlorine that is available after the chlorine demand is met is known as free available chlorine (White, 1999). This is all the chlorine that is not bound; hence it is available to react with other sources of bacteria or contaminants. It may be in the form of dissolved gas (Cl_2), hypochlorous acid (HOCl), or hypochlorite ion (OCl^-), but does not include chlorine combined with an amine (ammonia or nitrogen) or other organic compound (White, 1999). Total chlorine is defined as the sum of the free available chlorine and the bound chlorine (chlorine that is bound with organic and inorganic matter (White, 1999). Bound chlorine is inactive and unable to exert any disinfecting action.

Turbidity of the chill water has been investigated (Mead and Thomas, 1973; Tsai et al., 1992; Yang et al., 2002). The amount of suspended organic matter was measured using turbidity and found to be increasing with the increasing number of carcasses passing through the chill tank and with the decreasing percentage of free residual chlorine present (Mead and Thomas, 1973). The disinfecting action of chlorine was found to be affected by dissolved organic and inorganic matter, which reduced the availability of chlorine to act against microorganisms (Tsai et al., 1992).

Materials and methods

Sample size

The parameters were measured during sample collection in order to assess *Salmonella* prevalence in pre-chill carcass rinse samples and to assess the strength of association between post-chill prevalence and other parameters such as pre-chill *Salmonella* levels, pH, chlorine, turbidity, water flow rate, and temperature. As such, the prevalence was considered when estimating the required sample size. The calculated sample size for a two sided test, assuming a pre-chill prevalence of *Salmonella* of 50%, an alpha level of 0.05, and power of 95% was 401 samples to estimate the true prevalence within 9 percentage points (Selvin, 1996). The pre-chill prevalence of 50% was selected for the sample size calculation because the number of samples needed increases as the estimated proportion approaches 50%.

Data collection

During seven trials done from June to September 2010, during which carcass rinse samples were collected, data of levels of HOCl, pH, temperature, turbidity and time of sample collection, were also gathered and recorded. A custom-assembled apparatus containing probes that measured chlorine, pH, temperature, and turbidity was connected to the chill tank via a siphon at a point closer to the exit. It was set to record readings of the various parameters every minute while the carcass rinse samples were being collected.

The data were saved onto the computer data logger, HOBO™ Data Logger Software. Turbidity was measured by Analite ™ NEP 9504GXI turbidity probe 400 NTU (McVan Instruments Pty. Ltd.,Columbus, Ohio). The temperature was measured by

Dulcotest Typ Pt 100 Dulcometer® DMT (ProMinent Dosiertechnik GmBH, Heidelberg Germany) and Dulcotest Typ PT – 1000 _SE Dulcometer® DMT (ProMinent Dosiertechnik GmBH, Heidelberg Germany). Chlorine was measured using the Dulcotest® Dulcotest Typ CLE 3-DMT 5ppm Chlorine sensor for free chlorine (ProMinent Dosiertechnik GmBH, Heidelberg Germany). The pH was measured by Dulcotest® Dulcotest Typ PHEX-112 for pH 1-12. The chlorine and pH probes were calibrated prior to each trial using HACH Test Kit (Pocket Colorimeter ™. Cat. No. 58700-12. A new water filter cartridge was used with each run to remove organic material that might interfere with the probes. An AquaPure AP801/ap801T Whole House Water Filter using Filter Cartridge AP814 with flow rate of 20 gpm (CUNO Incorporated, Meriden, CT) was used. Water was siphoned from the chill tank using 3/8 inch inside diameter tubing. Turbidity was measured prior to the filter. A water flow rate of 10 gpm was maintained through the filter and probe apparatus by electric pump SHURFLO® Model OEM 100-000-21 Pentair Water Model 100 Single Fixture Pump (Cypress, CA).

Statistical Analysis

Mixed-effects linear regression was done on data from trials one to seven using PROC MIXED of SAS V. 9.3 (SAS Institute, Cary, NC). The data from the seven trials was run with random effect of trial to account for the variation due to trial using the other values measured as fixed effects. The mixed procedure was run for the models turbidity=minute, HOCl=turbidity, and temperature=minute.

Model Formulation

Minute

In order to factor in the time of the recorded measurements, time was converted into Minute by the formula:

$$minute = (\frac{time}{60}) - 300 \quad 4.1$$

Where Time (in seconds) is divided by 60 to convert into minutes, and

300 is deducted because the processing plant begins operating at 5:00 a.m. or 300 minutes after midnight.

Dissociation Constant

The dissociation constant Ka is calculated by the formula:

$$K = 10 ** -\left(\left(\frac{3000}{CTemp} + 273\right)\right) - 10.0686 + (0.0253 * (CTemp + 273))) \quad 4.2$$

from (White, 1999) where CTemp is temperature in degrees Celsius.

Hypochlorous acid

Hypochlorous acid (HOCl) had to be recalculated with the following formula since the probe was measuring HOCl but assuming that the pH was 7.0.

Hypochlorous acid at pH 7

HOClpH7Pct or the percent hypochlorous acid at pH 7 is calculated:

$$HOClpH7Pct = \left(\frac{1}{1+\left(\frac{k}{10**-7}\right)}\right) \quad 4.3$$

Hypochlorous acid

$HOCl_5$ or Hypochlorous Acid was calculated by the formula:

$$HOCl = CCl * HOClpH7Pct \quad 4.4$$

Where HOCL is hypochlorous acid, or the free available chlorine; CCl is the measured chlorine level in the chill tank; and HOClpH7pct is the hypochlorous acid level recorded by the probe, assuming the pH is 7.0.

Hypochlorous acid at actual pH

HOClpHPct or the actual level of hypochlorous acid at actual pH

$$HOClCpHPct = \left(\frac{1}{1+\frac{K}{10**-CpH}}\right) \quad 4.5$$

Where HOClpHPct is the actual hypochlorous acid level; and CpH is the actual pH level in the chill tank;

Free chlorine

Free chlorine is calculated by the formula:

$$FreeCCl = \frac{HOCl}{HOClCpHPct}; \quad 4.6$$

Where freeCCl is the free chlorine level;

Results and discussion

The data was analyzed for relationships between the various parameters using mixed model analysis of variance. The intercept, coefficient estimates, standard error and p value for each regression mixed effects model are listed in Table 4.1. The results showed significant ($p<0.0001$) linear relationships between turbidity and minute,

temperature and minute, pH and minute and a significant ($p<0.0001$) negative linear relationship between chlorine (HOCl) and turbidity.

Table 4.1 Univariable fixed effects models

Outcome	Explanatory variable	Intercept	β	Std error of β	P-value
Turbidity	Minute	11.3	0.144	0.009	<0.0001
HOCl	Turbidity	1.4	-0.003	0.0005	<0.0001
Temperature	Minute	6.3	0.005	0.0004	<0.0001
pH	Minute	6.7	0.0001	0.00002	<0.0001

Turbidity and Minute

The turbidity of the chiller water was found to increase over time. This is as expected since as time goes on, more carcasses are added to the chiller and as a result, the level of organic matter accumulates in the chiller water, increasing the turbidity. The calculation for the conversion of time into minute was given previously (equation 4.1).

The fixed effects model for the relationship between turbidity and minute, with random effect of trial can solve for turbidity in the chiller water using the following equation:

$$Turbidity = 11.2576 + 0.1443 * minute. \quad 4.7$$

Turbidity had a significant positive relationship with minute ($p<0.0001$). As time increased, turbidity increased in the chiller water. This is the first statistical model of the relationship between turbidity and minute that was obtained from actual observed data

documented from the operations of a poultry processing plant. Mead and Thomas (1973) measured turbidity and number of carcasses, but not time. Yang et al (2002) measured age of chiller water (time in hours) and post-chill prevalence of *Salmonella* and *Campylobacter*, but did not measure turbidity.

Chlorine and turbidity

The results of the study showed a negative relationship between free available chlorine (HOCl) and turbidity. As turbidity increased the level of free available chlorine HOCl decreased. This is because higher turbidity levels indicate more organic matter in the chiller water which then binds the chlorine leaving less free available chlorine.

The fixed effects model for the relationship between free available chlorine (HOCl) and turbidity, with the random effect of trial can solve for the effective chlorine in the chiller water using the following equation:

$$HOCl = 1.4143 + (-0.00281 * Turbidity) \quad 4.8$$

The relationship between chlorine and turbidity has been studied by Tsai et al. (1992) and Mead and Thomas (1973), however, this study is the first to report the mathematical model of the relationship between turbidity and free available chlorine in an actual chill tank of a poultry processing plant. The negative linear relationship between turbidity and HOCl is significant $p<0.0001$, as turbidity increased, the free available chlorine (HOCl) decreased in the chiller water. The free available chlorine is important because of its bactericidal action. This action is diminished in the presence of increased turbidity.

Temperature and minute

The temperature of the chiller water was found to increase with time. The fixed effects model for the relationship between temperature and time, with the random effect of trial can solve for the temperature of the chiller water using the following equation:

$$Temperature = 6.2747 + 0.004768 * Minute \quad 4.9$$

The positive relationship between temperature and minute was significant also ($p<0.0001$). This highlights the importance of monitoring and maintaining the temperature in the chiller. The temperature of the chiller water is important for the chilling effect which also reduces microbial load, as well as the effect of temperature on the ionization constant (K_i) which is important in chlorine hydrolysis. Table 4.2 shows the minimum, maximum and mean values of the parameters measured in the study.

Table 4.2 Minimum, maximum and mean values of the parameters

Variable	N	Mean	Standard deviation	Minimum	Maximum
pH	614	6.8	0.14	6.60	7.4
Turbidity	557	103.8	41.40	1.1	219.0
HOCl5	614	1.0	0.57	0.04	1.9
Temperature	614	9.1	1.81	5.72	17.0

The relationships between turbidity and time, pH and time, and temperature and time in the water immersion chill tank are interesting results and would be useful in further modeling studies. It is also important to note that the parameter readings were actual field situation observations, taken during normal operations of the processing plant, using real time and real production processing as opposed to experimental situations.

The quantitative measurements of these variables over time in the water immersion chill tank provide regression models that elucidate the interplay between these factors that influence *Salmonella* contamination in the chill tank. The results show that as time increases, the level of turbidity, pH and temperature in the chiller water also increase correspondingly. On the other hand, as the turbidity level rises, the level of effective chlorine (free available chlorine) decreases. As the free available chlorine decreases in the chiller water, the bactericidal effect of chlorine is diminished, leading to an increase in post-chill *Salmonella* prevalence. The data obtained from this research provides a valuable contribution to the understanding of what is happening in the chill tank of the poultry processing plant, and demonstrates the relationships between these factors. Greater knowledge of these relationships is important in managing the water immersion chill tank and interventions to reduce the *Salmonella* contamination.

Conclusions

The specially designed apparatus consisting of probes for pH, temperature, turbidity, and chlorine connected to a data logger proved to be effective in collecting the data needed for the study. The said apparatus could be further modified according to the needs of the processing plant companies as a monitoring tool.

The study was able to obtain statistically significant relationships among the factors associated with the chill tank of the processing plant. There was a statistically significant linear relationship between turbidity of the chiller water and time ($p<0.0001$). There was a significant linear relationship between pH and time ($p<0.0001$) and between temperature and time ($p<0.0001$). There was a statistically significant negative linear relationship between turbidity and effective chlorine ($p<0.0001$).

The relationships of these factors with each other also affect free available chlorine. Since in the chill tank of the processing plant, free available chlorine is important for its bactericidal activity and in reducing *Salmonella*, then the relationships also ultimately influence *Salmonella* contamination of the broiler carcasses particularly their status when they exit the chiller.

Linear regression equations for predicting the dependent variables (turbidity, temperature, pH, effective chlorine) were derived from actual field observations on these parameters during normal operation of a poultry processing plant and provide real insights. These models are valuable to the industry knowledge base on the relationships of temperature, turbidity, and chlorine in the chiller water of a poultry processing plant.

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CHAPTER V

A DATA-BASED SYSTEM DYNAMICS MODEL TO SIMULATE *SALMONELLA* CONTAMINATION OF BROILER CARCASSES IN THE CHILL TANK OF A POULTRY PROCESSING PLANT

Abstract

A system dynamics model to simulate *Salmonella* contamination of poultry broiler carcasses in the chill tank of a poultry processing plant was developed using the Structure Thinking, Exponential Learning Laboratory with Animation (STELLA® STELLA program and was also validated. The model is based upon actual data collected from a poultry processing plant in Mississippi. Pre-chill and post-chill prevalence of *Salmonella* contamination was determined by whole carcass rinse, and compared with recorded measurements of levels of chlorine, turbidity, pH and temperature collected from the water immersion chill tank. A cross-contamination probability model was developed:

$$P = [\frac{1}{1+e^{-y}}] \quad 5.1$$

where *P* is the probability that a single broiler carcass exits the chill tanks positive for *Salmonella* contamination, and *y* is a function of post-chill *Salmonella* incidence and free available chlorine (hypochlorous acid or HOCl), as well as the interaction of both.

Also, a decontamination probability model was developed :

$$P = 1 - \left\{\frac{1}{[1+e^{-z}]}\right\} \quad 5.2$$

where P is the probability that a single broiler carcass exits the chill tank negative for *Salmonella* contamination, and z is a function of post-chill *Salmonella* cumulative incidence and free available chlorine.

The model shows great potential for predicting cross-contamination and decontamination in the chill tank, and could be a powerful tool for testing pathogen interventions to be used in poultry processing plants

Key words: Salmonella, system dynamics model, chill tank

Introduction

Managing risks related to product caused illnesses is a major focus of the modern 21st century food production industry, with pathogens identified as the main cause of foodborne disease. The food animal industry considers consumer safety threats of the highest order of importance and has worked to address this issue. Poultry ranks as the highest amount of meat products consumed at nearly 100 lbs. per capita (Council, 2013). *Salmonella* contamination of broiler carcasses continues to be a challenge for the poultry processing industry.

Salmonella infections are the most common bacterial foodborne infection, and the most common cause of hospitalization and death tracked in the FoodNet for the past 15 years (CDC, 2011). The Food Safety Inspection Service (FSIS) monitors regulatory compliance relative to the *Salmonella* performance standard of a broiler processing facility after the final intervention, which is usually as the carcasses exit the chill tank. (Federal Register, 1996; USDA, 2010a). The chill tank of the poultry processing plant

has been identified as one of the sites for cross-contamination of broiler carcasses (Lillard, 1990; Yang et al., 2002). Research has shown that *Salmonella* growth is affected by various factors including chlorine level, temperature, pH of the chiller water and pre-chill prevalence (Foster, 1991; Koutsoumanis et al., 2004; Lillard, 1980; Membre et al., 2005). In the past few decades, various modeling techniques have been applied to the study of *Salmonella* growth and contamination particularly predictive microbiology models (Dalgaard et al., 2002; Koutsoumanis et al., 2004; McClure et al., 1994). Classic predictive models describe the effects on *Salmonella* growth as a result of changes in certain parameters or factors in the chiller system like pH and temperature of the water and other components. Kinetic models add the dimension of time. Yang et al. (2002) developed a cross-contamination probability model which went a step further, in which it looked at the interactions between different individual parameters. However, these types of dynamic models lack the feedback loop and the ability to model non-linear relationships that are seen in actual biological systems. System dynamics models have the advantage of the feedback loops and the flexibility to model some of nature's more complex relationships (Forrester, 1961; Hannon and Ruth, 1997). System dynamics has been applied in other biological systems (Elshorbaggy, 2005; Hannon and Ruth, 1997). Using the system dynamics approach, *Salmonella* contamination in a broiler processing plant chill tank would be viewed as a system of factors linked to each other through a series of direct and indirect relationships and some type of feedback mechanism. This is in contrast to a simple linear relationship that cannot accurately represent the complexity that exists in actual biological systems.

System dynamics and simulation modeling

The system dynamics (SD) approach was initially proposed by Jay W. Forrester (Forrester, 1961) and is an alternative view on management and heuristics. System dynamics modeling looks at the interrelationships between the various factors involved in a process. In system dynamics modeling, the telephone is not just the mere assembly of wires and modems but the interrelationships between these components (Forester, 1960). In like manner, the purpose of this work was to obtain a better understanding of the broiler chilling system, i.e., the *Salmonella* contamination of the chicken carcasses in the chill tank, and the interrelationships between the factors involved. System dynamics modeling allows the inclusion of feedback loops which are important in the understanding of the complex relationships that exist in nature between the factors and the system as a whole. Computer simulation modeling is one of the techniques in systems engineering that has helped to revolutionize such things as the design and management of manufacturing systems, telecommunication networks, banking, marketing, and aquaculture. Further, this technique may provide not only the basis for identifying optimal management practices but also a practical tool that can be used daily in manufacturing and processing (Halachmi et al., 2005). Hannon and Ruth (1997) stated that models have various advantages and functions and allow understanding of the mechanisms at work. By using models instead of real systems, alternative operations or scenarios could be attempted without incurring the real-life losses that could occur if the alterations were done on the real system. System dynamics models do these operations with the additional advantage of allowing a look at interrelationships between factors, circular relationships and feedback phenomena.

System dynamics approach modeling using STELLA ® Software program has various advantages including being user-friendly and intuitive (Elshorbaggy et al., 2005). Available data may be easily used, and can be expanded as more data becomes available (Elshorbaggy et al., 2005). The model developed is dynamic and can be adjusted to represent feedback mechanisms. Using this program, modeling of various scenarios can be done easily (Elshorbaggy, 2005; Hannon and Ruth, 1997). STELLA® Software is one of the more popular commercially available simulation modeling programs. It is very easy to learn and apply, even without knowledge of programming language. It makes use of icons such as reservoirs, spigots, converters, flow converters, graphs, and tables (Richmond, 1991). The need for realistic, user-friendly models that can copy complex systems has been highlighted in other fields (Elshorbaggy, 2005). Similarly, there is potential for such models in the poultry processing industry, particularly those that could help managers in making decisions with regards to risk assessment and enteric pathogen mitigation. This current research will help fill knowledge gaps and uncertainties with regards to what is happening in the chill tank.

In this research, factors that affect *Salmonella* contamination in the chill tank will be studied to develop the model. These factors are pre-chill *Salmonella* prevalence, pH, temperature, level of hypochlorous acid, and turbidity of the chill tank water. These factors, which have a significant relationship with the post-chill prevalence of *Salmonella* contamination, will be characterized and these relationships will be used to develop the system dynamics model of *Salmonella* contamination at the level of the chill tank.

The role of pre-chill prevalence in *Salmonella* contamination has been studied. A high level of *Salmonella* contamination of the chicken carcass as it comes into the chill

tank contributes to increasing the level of *Salmonella* found in the chill tank and be available to contaminate the chicken carcasses that enter the tank; the bacteriological quality of the water in the chill tank is dependent on the level of contamination of the chicken carcasses entering the chill tank (Bailey et al., 1987; Mead, 1989; Yang et al., 2002).

The pH of its environment affects *Salmonella* growth and destroys the pathogen at high levels (Sampathkumar et al., 2003; Teo et al., 1996), while low pH can stimulate the acidity tolerance response (ATR). In addition, pH affects the dissociation of hypochlorous acid (HOCl), which is the bactericidal component of chlorine (White, 1999).

Chlorine is a disinfectant commonly used in water systems (Cole, 1987) which has been used in immersion chillers to reduce *Salmonella* levels on broiler carcasses (Magwood, 1967; Mead and Thomas, 1973). Temperature and pH determine the relative proportions of the free chlorine forms (Edstrom Industries Manual, 2003). Chlorine is most effective at pH 5 to 7, where HOCl is predominant because HOCL is 100 times more powerful as an oxidant and disinfectant than the hypochlorite ion (White, 1999). In addition to temperature and pH, organic matter also affects the activity of chlorine and the survival of bacteria in the chiller water (Bailey et al., 1987; Mead, 1989; Yang et al., 2001). The amount of chlorine added, the presence of organic matter, and the contact time have been found to influence the residual free chlorine in chilled water (Tsai, 1992). Yang et al(2001) found that aging the chilled water reduced the residual free chlorine levels and also reduced the effect of chlorine in reducing *Salmonella* contamination.

This research will look at these factors in an actual operating processing plant chill tank and analyze how they relate to one another as well as with the *Salmonella* contamination of broiler carcasses. The system dynamics model will be constructed to model these relationships, and aid in the understanding of the *Salmonella* contamination of broiler carcasses in the chill tank of the processing plant.

Model validation

Validation can be considered as one of the most important parts of the modeling process since it determines the practicality of the model that has been developed. Solberg (1992) said that the validity of a model determines its power. Model validation has been found to be more problematic for system dynamics models including STELLA® (Martis, 2006; Mohapatra, 1987).

Several model validation techniques have been developed (Barlas, 1989, 1996; Forrester and Senge, 1980; Khazanchi, 1996; Martis, 2006). For Forester and Senge (1980) a model is validated by its objective, structure, behavior and policy implications. The model is subjected to tests for the model structure and behavior which consist of questions about the structure and behavior and how they relate to the real system being modeled. For example, model structure is tested for suitability by checking the compatibility of the model structure with the real system, and its ability to withstand shocks applied to it. Model structure consistency is validated by the face validity test – how similar is the model structure to the system being modeled and by questions referring to consistency of the parameters used in the model and in reality. Model structure usefulness is tested by the appropriateness of the model to the audience for the study. Model behavior is similarly validated according to suitability, consistency and

usefulness. For example, for suitability of model behavior, sensitivity analysis may be done to check if the model is sensitive to variations. Khazanchi (1996) developed a model validation scheme that is more applicable to qualitative models and consists of eight questions about the model, namely: "is the model plausible, reasonable, feasible, effective, pragmatic, empirical, predictive, and certifiable ?" (Khazanchi, 1996).

Another technique is face validity. Simply put, this technique seeks the opinion of experts to determine if the system model behavior is reasonable (Forrester, 1961; Law and Kelton, 1982; Naylor and Finger, 1967). After a model has been formulated and run, there is a need to validate it. To validate is to answer the question "how representative of reality is the model?" (Naylor and Finger, 1967).

A three step approach to model validation was developed by Naylor and Finger (1967) and modified by Law and Kelton (1982). The steps are 1) begin with a model with high face validity, 2) perform empirical testing, and 3) determine how representative the simulation output data are. A model with high face validity is one that is accepted as a reasonable representation of the system by experts in the field (Law and Kelton, 1982). The second step, empirical testing may be carried out by comparing observed data with literature data (Khazanchi, 1996; Law and Kelton, 1982). The third step is testing the "representative-ness" of the simulation data. Actual observed data are compared with model predicted data, and the predicted data should be very close to the reality (Law and Kelton, 1982).

Objectives of the study

Salmonella contamination of broiler carcasses is a threat to the health of individual consumers, to food safety, and to the livelihood of millions of families who are

employed by the poultry industry. Although much has been learned about the microbiology of *Salmonella*, there are still gaps of knowledge in its ecology, particularly the relationships between the parameters involved with *Salmonella* contamination in the chill tank stage of the processing. System dynamics modeling has not been used in risk modeling for the poultry processing industry. In addition, no studies in actual chill tanks in commercial processing plants have been done that comprehensively included determination of changes in the six parameters namely 1) pH, 2) temperature, 3) chlorine concentration and 4) turbidity level in the chill tank water, 5) pre-chill and 6) post-chill *Salmonella* contamination status of the broiler carcass, and the relationships between these parameters over time. Developing a system dynamics simulation model for *Salmonella* contamination at the chilling stage of poultry processing will provide a powerful tool for assessing the risk of *Salmonella* contamination in the chiller as well as for implementing intervention strategies. The objective of this research is to develop a system dynamics model of *Salmonella* contamination of broiler carcasses in the chill tank of a poultry processing plant that is able to predict the probability of a single broiler carcass exiting the chill tank as positive using actual pre-chill and post-chill prevalence data from whole carcass rinse samples and recorded measurements of turbidity, pH, and chlorine levels in a commercial poultry processing plant in Mississippi.

Materials and methods

Sample size calculations

Accurately assessing *Salmonella* prevalence in pre-chill carcass rinse samples and assessing the strength of association between post-chill prevalence and other parameters such as pre-chill *Salmonella* levels, pH, chlorine, turbidity, water flow rate, and

temperature were considered when estimating the required sample size. The calculated sample size for a two sided test, assuming a pre-chill prevalence of *Salmonella* of 50%, an alpha level of 0.05, and power of 95% was 401 samples to estimate the true prevalence within 9 percentage points (Selvin, 1996). The pre-chill prevalence of 50% was selected for the sample size calculation because the number of samples needed increases as the estimated proportion approaches 50%.

Sample collection at the processing plant

A total of seven trials were conducted in a commercial poultry processing plant, and the broiler carcasses were sampled according to the method determined in another study (Chapter 2), the adjacent carcass method. The pre-chill carcass was rinsed and returned to the processing line while the adjacent carcass was tagged, but not rinsed, and returned to the processing line. As the tagged carcasses exited the chill tank, a carcass rinse was taken. The carcass rinse samples were transported to the laboratory and tested for the presence of *Salmonella*.

As the broiler carcasses moved through the chill tank, the conditions of the water in the chill tank were monitored for pH, chlorine, turbidity, and temperature levels. A set of sensors (probes) were packaged in an apparatus that siphoned water from the chill tank. As the water passed through the siphon pipe, the sensors measured the pH, chlorine, turbidity, and temperature. The information from the sensors was recorded by a data acquisition system for later downloading into a portable laptop computer. The instrumentation system was designed for fast installation and fast uninstalls to allow it to be portable to different processing plants. The data was sampled at a rate of approximately two samples per minute or less to allow for the possibility of data

decimation (reduction) as well as data integration (averaging). The time was recorded as each carcass rinse was performed so that the chill tank water parameters could be matched temporally with carcass rinse sample collection.

Microbiological methods

Whole carcass rinse method

Using fresh latex gloves for each carcass, the whole broiler carcass was removed from the processing line and placed inside a sterile plastic bag with 100 ml of Butterfield's solution, and shaken for one minute as previously described (Cox et al., 1983). The rinse sample was collected in a sterile plastic bottle, properly labeled Bn (for pre-chill whole carcass rinse) or Cn (for the post-chill whole carcass rinse), and stored and transported to the laboratory on wet ice.

***Salmonella* testing**

The samples were tested for the presence of *Salmonella* using the procedure according to Rybolt et al. (2004) which is a combination of selective enrichment in tetrathionate broth followed by Rappaport-Vassiliadis broth. This method has been shown to be more effective in detecting positive samples. The protocol (Rybolt et al., 2004) for the method is as follows:

1. After initial collection and preparation as described above, incubate samples 18-24 hours at 42°C.
2. Transfer 1.0 ml from the rinse solution to 9.0 ml of Tetrathionate broth, and incubate 18-24 hours at 42°C.

3. Transfer 0.1 ml to 10.0 ml of Rappaport-Vassiliadis broth, and incubate 18-24 hours at 42°C.
4. Plate to XLT4 and incubate 18-24 hours at 37°C.
5. Suspect colonies used to inoculate TSI and LIA slants, incubate 18-24 hours at 37°C.
6. Read slants after incubation, positive reactions for *Salmonella* tested against anti-*Salmonella* anti-sera for confirmation.

Collecting data on temperature, pH, free available chlorine, turbidity

To measure temperature, pH, free available chlorine, and level of organic matter in the chill water a specially assembled apparatus consisting of various probes and a siphon was placed at a point after the entrance end of the chill tank. The siphon, made of PVC pipe, collected water sampled from the tank, which then ran through the various probes in the apparatus, namely sensors that measure temperature, pH, free available chlorine, and turbidity. Simultaneously, the readings for temperature, pH, free available chlorine in the form of hypochlorous acid (HOCl), turbidity in the chill tank, were recorded including the time. The readings from the probes were automatically recorded by a computerized data logger (Hobo®Data Loggers, Southern, MA). Each of the probes were calibrated prior to running the apparatus at every trial. The data was transferred from the data logger software to spreadsheet software (Microsoft ® Excel 2010 version, Redmond, WA) and then exported to a statistical analysis program (SAS ® 9.3, SAS Institute Inc., Cary, NC).

The following is the list of the probes and sensors used in the apparatus:

1.Turbidity was measured by Analite ™ probe (Columbus, Ohio).

2. pH and temperature were measured by Dulcometer® DMT (ProMinent Dosiertechnik GmBH, Heidelberg Germany).

3. Chlorine was measured using the Dulcotest® Chlorine sensor for free chlorine (ProMinent Dosiertechnik GmBH, Heidelberg Germany).

Data analysis

PROC GLIMMIX in SAS v 9.3 (SAS Institute, Cary, NC) was used to develop logistic regression models assessing the association between the presence or absence of *Salmonella* and explanatory variables of free available chlorine level of chiller water, post-chill *Salmonella* incidence, and their interaction. The data for all the trials were considered initially as one set of data and with no random effect of trial. Considering the data as one set of data allowed the data to be treated as if all the data were obtained during one day of processing, to maximize the use of the data available for developing the model. A logistic regression model, designated the cross-contamination model, was developed using data restricted to broiler carcasses that were *Salmonella* negative at pre-chill. A second logistic regression model, designated the decontamination model, was developed using data restricted to broiler carcasses that were *Salmonella* positive at pre-chill.

System dynamics modeling

The *Salmonella* contamination of broiler carcasses in the chill tank of a poultry processing plant was modeled using a system dynamics modeling program (STELLA® 8.1.1, Iseesystems, Inc., Lebanon, NH). A literature based model described in Chapter 3 served as the foundation for the model developed and described here. Relationships

between various parameters in the chill tank, described in Chapter 4, were utilized in this model and replaced many of the literature based relationships used in the model presented in Chapter 3.

Results and discussion

Salmonella prevalence results

The results of the tests for the presence of *Salmonella* in the pre-chill and post-chill whole carcass rinses for all the trials are summarized in Table 5.1. The average pre-chill prevalence for all the trials in the study was 25%. The average post-chill prevalence was 8%. Trial 2 had the least pre-chill percent prevalence (4.4%), and trial 4 had the highest (74.75%). Trial 7 had the least post-chill percent prevalence (0%) and Trial 5 had the highest (30.49%).

Table 5.1 *Salmonella* prevalence results

Trial	PRE-CHILL PREVALENCE (%)	POST CHILL PREVALENCE (%)
1	5/63 (7.9)	4/63 (6.3)
2	4/91 (4.4)	1/94 (1.1)
3	6/95 (6.3)	1/93 (1.1)
4	74/99 (74.7)	18/94 (19.2)
5	33/81 (40.7)	25/82 (30.5)
6	20/92 (21.7)	1/90 (1.1)
7	20/97 (20.6)	0/93 (0.0)

In all the trials, the post-chill prevalence was lower than the pre-chill prevalence. This is in accordance with what would be expected due to the effect of chilling and chlorine on *Salmonella* in the chiller. The chilling process reduces the temperature of the chicken carcasses and also delays or prevents the growth of bacteria (Mead 1989, ICMSF 1998). The use of chlorine in immersion chillers has been found to reduce *Salmonella* levels on broiler carcasses (Magwood, 1967), (Mead, 1973), (Mead, 1994). There is also the added confidence that whole carcass rinse did not influence the post-chill result since the adjacent pair sampling method, as described in Chapter 2, was used. Table 5.2 shows the mean and range of values of the pH, turbidity, hypochlorous acid (HOCl), and minutes recorded during this current study.

Table 5.2 Summary of mean, minimum and maximum values of pH, turbidity and hypochlorous acid from trials 1-7

Variable	N	Mean	Standard deviation	Minimum	Maximum
pH	614	6.8	0.14	6.6	7.4
Turbidity	557	103.8	41.40	1.1	219.0
HOCl5	633	1.4	0.935	0.07	4.3
Temperature	614	9.1	1.8	5.7	17.0

Model development and formulation

Cross-contamination probability model

The probability model

$$P = [\frac{1}{1+e^{-y}}] \quad 5.1$$

has been used to determine the probability (P) of a single spore initiating growth, germinating, and forming toxin from *Clostridium* and *Campylobacter*, where Y is a

polynomial function of environmental factors (Roberts et al., 1981; Roberts and Jarvis, 1983; Skjerve and Brennhovd, 1992). The probability P has a value between 0 and 1. The bigger the value of *Y*, the more *P* approaches 1. On the other hand, if Y is a bigger negative value, the term e^{-y} becomes large and *P* becomes closer to 0.

Yang et al (2002) adapted the probability model to predict the probability of a single chicken drumstick contaminated by *Campylobacter jejuni* or *Salmonella typhimurium*, where Y was a polynomial function of chlorine level (Cl), pre-chill incidence (PRI), water age (Age) and the cross-terms of Cl x PRI, Cl x Age, PRI x Age and PRI x Age x Cl.

In this current study, the probability model was adapted to predict the probability of a single broiler carcass exiting the chill tank as positive for *Salmonella* contamination when it was negative at pre-chill:, where Y is the logit function of the level of the hypochlorous acid (HOCl5) at the time the carcass entered the chill tank, post-chill cumulative incidence (CCumCt) and the cross-terms of HOCl5* CCumCt, for samples that were negative at pre-chill.

The probability model was further adapted to predict the probability of a single broiler carcass exiting the chill tank as negative for *Salmonella* contamination when it was positive at pre-chill: where Z instead of Y is the logit function of effective chlorine, post-chill cumulative incidence and the cross terms effective chlorine * post-chill cumulative incidence for samples that were positive at pre-chill.

The coefficients of the explanatory variables, standard error and p value are listed in Table 5.3 for the cross-contamination model. The Y model can predict the log odds

that a broiler carcass that was negative when it entered the chill tank would exit the chill tank cross-contaminated with *Salmonella*.

Table 5.3 Coefficients of Y in the cross-contamination model

Effect	Estimate	Standard Error	Pr > t|
Intercept	2.4	1.48	0.1062
HOCl	-3.7	1.56	0.0167
Post-chill incidence	-0.2	0.06	<.0001
HOCl* Post-chill incidence	0.1	0.05	0.0059

The probability of a broiler carcass that is negative for *Salmonella* becoming positive was found by the cross-contamination probability model:

$$P = [\frac{1}{1+e^{-y}}] \quad 5.1$$

The results show that indeed chlorine level of the chiller the water had an inverse linear relationship with the *Salmonella* contamination of chickens exiting the chiller. The decontamination model can predict the log odds that a positive broiler carcass entering the chill tank would exit the chill tank positive for *Salmonella* contamination. The coefficients of the explanatory variables in the decontamination model, standard of error and p value of the estimates are listed in Table 5.4.

Table 5.4 Coefficients of Z in the decontamination model

Effect	Estimate	Standard Error	Pr> | t |
Intercept	0.87	1.48	0.5560
HOCl	-1.64	1.38	0.2363
Post-chill incidence	-0.11	0.06	0.0700
HOCl x Post-chill incidence	0.07	0.05	0.1510

The probability of a broiler carcass that is positive for *Salmonella* becoming negative was found by the decontamination probability model:

$$P = 1 - \left\{\frac{1}{[1+e^{-z}]}\right\} \qquad 5.2$$

System dynamics model of *Salmonella* contamination at the chill tank of a broiler poultry processing plant

In this current study, *Salmonella* contamination of broiler carcasses within the water immersion chill tank of the poultry processing plant is conceived as a system, with various factors whose interactions and relationships contribute ultimately to the endpoint *Salmonella* contamination status of an individual broiler poultry carcass. Viewed as a system, the *Salmonella* contamination of poultry broiler carcasses in the chill tank of the processing plant is composed of the following processes and factors: pre-chill prevalence of *Salmonella*, flock size, level of chlorine in ppm, level of turbidity, temperature, level of pH, and length of time in the chill tank.

Figure 5.1 shows the System Dynamics (SD) Model of *Salmonella* contamination in the water immersion chill tank in the poultry processing plant. In STELLA® program, the double line arrows show the flow of the chickens from a source represented by a cloud or a stock (rectangle), into another stock or cloud. The single lines connect objects (coefficients or processes) together. These are called connectors, which show the flow of information inside the model. Converters (such as flock size and temperature) can represent any variable as a function of time or any input value or parameter.

Using the STELLA™ simulation modeling program, a system dynamics model of *Salmonella* contamination of broiler poultry carcasses in the poultry processing plant, at the level of the chill tank is constructed.

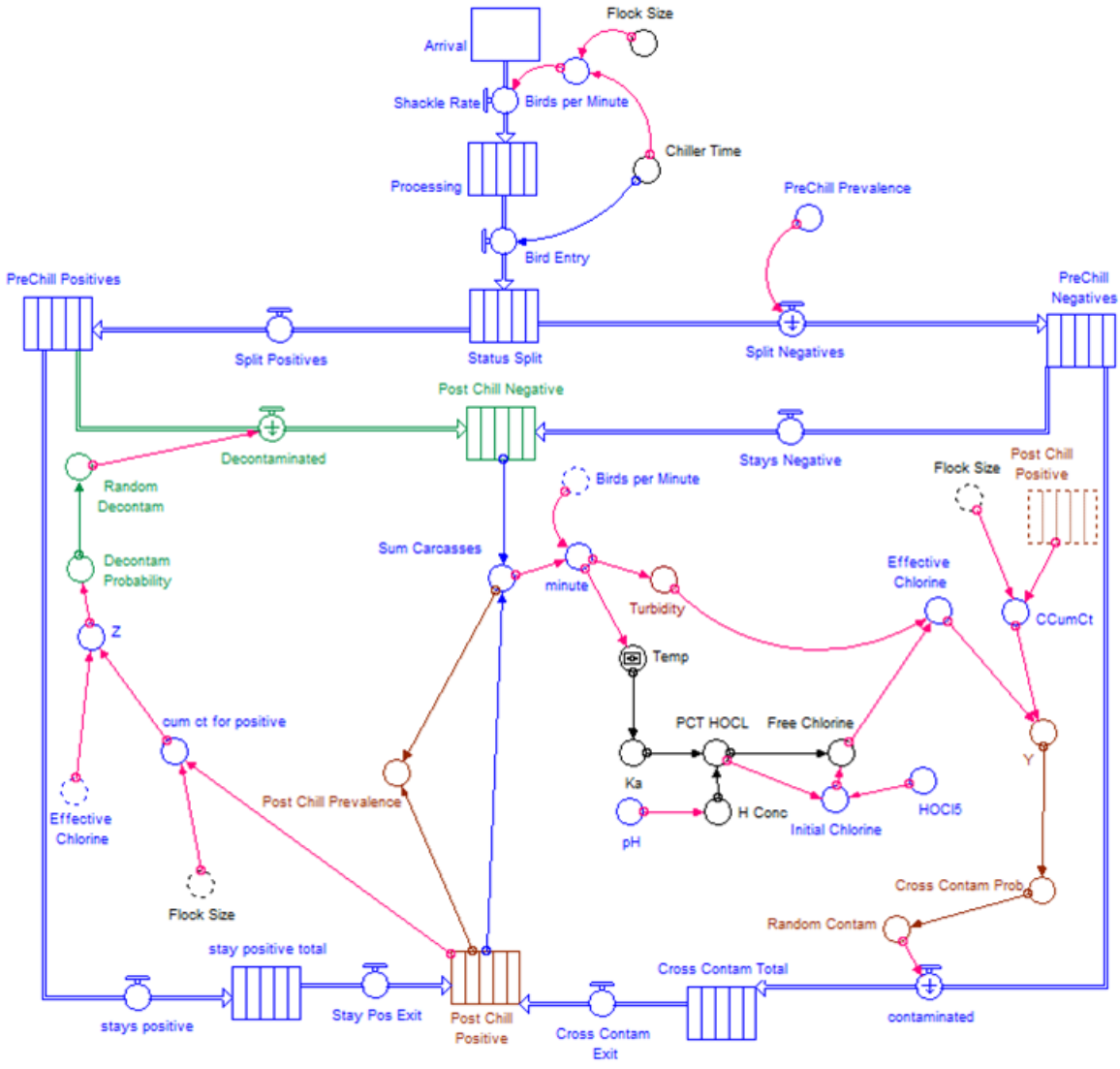

Figure 5.1 System dynamics model of *Salmonella* contamination in the poultry processing plant at the level of the chill tank

The SD Model of *Salmonella* contamination in the poultry processing plant chill tank begins with the arrival of the birds. As the birds enter the processing plant, factors such as the flock size of the arriving batch, the speed of the line (in birds per minute) affect the flow of the birds through processing (prior to entry into the chiller). After initial processing, the chiller time (or time the birds take to go through the chiller) is an important input. As the birds enter the chill tank, the model splits into two ways. Based on the pre-chill prevalence (no. of positive for *Salmonella*/samples tested at the pre-chill stage), the broilers are stochastically split into either positive or negative. Those that were positive based on the pre-chill prevalence would flow through the split positive flow onto the pre-chill positive stock on the left side of the model, while those that were negative, based on the pre-chill prevalence, would flow through the split negative flow onto the pre-chill negative stock on the right side of the model. In the model, the pre-chill prevalence can be inputted into a slider and the split conveyor calculates how many carcasses should flow into the split negative and split positive based on the level of pre-chill prevalence. Note that whether or not they are split positive or split negative, all these carcasses have now entered the chill tank.

Within the chill tank, the broiler carcasses could either be cross-contaminated or decontaminated. Yang et al. (2002) showed a cross-contamination probability model for experimental drumsticks using Y made up of a polynomial function of pre-chill incidence, age of chiller water and chlorine level. In contrast, in this current study, the pre-chill incidence was used as a factor for the cross-contamination model. However, it was found to be an important variable input in the model. As the broiler carcass entered

the chill tank, it carried with it the pre-chill prevalence for the trial. Thus, depending on the pre-chill prevalence, the broiler carcass is stochastically directed as described earlier.

In the chill tank, two probabilities exist for each pre-chill stock. For the pre-chill negative stocks, the probability that the pre-chill negative becomes cross-contaminated and exits the chill tank as post-chill positive (cross-contamination probability model) or it stays negative and exits the chill tank as post chill negative. For the pre-chill positive stocks, the two possibilities are that the pre-chill positive broiler carcass stays positive and exits the chill tank post-chill positive for *Salmonella* or that the pre-chill positive becomes decontaminated in the chill tank and exits as post chill negative (decontamination probability model).

Those carcasses that stay positive or negative are represented by the "stay positive" and "stay negative" flows. The hydrolysis of chlorine is represented within the chill tank to symbolize that it is occurring in the chiller water. The process is influenced by pH, temperature, the dissociation constant Ka, and the initial chlorine level. The temperature affects the dissociation constant Ka, hence there is an arrow in that direction. The pH affects the ionization of Hydrogen. The Ka and the hydrogen ions both affect the percent HOCl, thus there are arrows from both to the HOCl. The percent HOCl and the initial chlorine both affect the effective chlorine which is the free available chlorine.

Cross-contamination probability model

The cross-contamination model Y is placed after the pre-chill negative stock, and input as the converter Y. The model was a logit function of level of effective chlorine, post-chill cumulative incidence, and the interaction of effective chlorine level and post-chill cumulative incidence. Post-chill cumulative incidence is the cumulative number of

broilers that became or remained positive for *Salmonella* as carcasses progressed through the chill tank.

Effective chlorine

Effective chlorine was a function of initial chlorine and turbidity. The regression equation is:

$$\text{Effective chlorine} = \text{free chlorine} + ((-0.00445)^* (\text{turbidity})) \quad 5.3$$

Turbidity

Turbidity was a function of time (minute). The regression equation is:

$$\text{Turbidity} = -1.8573 + (0.19 * \text{minute}) \quad 5.4$$

The value of Y was then used as an input into the "cross contamination probability converter" which used equation 5.1 to provide a probability of a carcass that was *Salmonella* negative at pre-chill would become positive. This probability was then used in the random contamination converter to stochastically determine the leakage fraction in the contaminated leakage flow which determined the number of pre-chill negative carcasses that became post-chill positive and the number that remained negative.

Decontamination probability model

The decontamination model Z is placed after the pre-chill positive stock and input into the converter Z. The model was a logit function of effective chlorine, post-chill cumulative incidence and the interaction of both, as described for the cross-contamination model.

Similar to the cross-contamination flow of the model, the value of Z was used as an input into the "decontamination probability converter" which used equation 5.2 to provide a probability of a carcass that was *Salmonella* positive at pre-chill would become negative. This probability was then used in the random decontamination converter to stochastically determine the leakage fraction in the decontaminated leakage flow which determined the number of pre-chill positive carcasses that became post-chill negative and the number that remained positive.

Other insights from the model

Although the pre-chill prevalence is not in the probability model, it is still an important input in the system dynamics model. If the pre-chill prevalence could be reduced, whether from the farm level or at the plant level, it could help reduce the cross-contamination in the chill tank. The relationship between time, turbidity and effective chlorine is highlighted in the model. Time is directly proportional to turbidity, i.e. increase in time results in an increase in turbidity. This is because over time, the number of broiler carcasses that enter the chill tank also increases, thereby increasing organic matter load. There is an inverse relationship between turbidity and effective chlorine. The increase in turbidity leads to a corresponding decrease in effective chlorine. With higher turbidity, there is more organic matter in the chill tank, and these organic compounds bind with the chlorine leading to the lower amount of effective chlorine that is able to kill *Salmonella*. The relationship between pH and effective chlorine is very important because at pH higher than 7.0, chlorine becomes inactive as a disinfectant. Maintaining the pH of the chill tank between 6 and 7 is the best. At pH higher than 7, the dissociation favors the formation of hypochlorite ions which is not highly disinfectant.

At pH below 6, a higher proportion of the chlorine becomes gaseous and may, depending on levels, be irritating to workers and an occupational health hazard (White, 1999).

The system dynamics model of *Salmonella* contamination at the level of the chill tank provides a better understanding of the interactions between these factors that comprise this system. This user-friendly simulation model is a powerful tool for managers and regulators in testing interventions to be used in poultry processing plants. It would be more economical to simulate changing any of the settings like the pH, the chlorine level, using the simulation model rather than encountering the financial and production challenges of running the actual intervention.

The complete mathematical formulations for the model are listed in Appendix A

Model validation

Face validity test

One of the main methods for validation of a model is the face validity test (Forester and Senge, 1980). This is simply the degree, to which the model structure resembles the real system, i.e. is it a reasonable representation of the system being modeled.

Looking at the SD Model of the *Salmonella* contamination of broiler carcasses at the chill tank of a poultry processing plant, it begins with the arrival of the birds at the plant, and the size of the flock that has arrived for processing. This step is followed by the shackling of the birds onto the conveyor for processing, including a variable for the speed of the line and the rate at which the birds are taken up into the line. The next step, "bird entry" signifies the entry into the chill tank. The chiller time or the time it takes for the broiler carcass to go through the chill tank is input into this step. In the next step, the

"status split", the pre-chill prevalence is the variable that would determine how many of the broiler carcasses enter the chill tank as positive, or as negative for *Salmonella*. Pre-chill positive or negative carcasses may either be decontaminated or cross-contaminated within the chill tank and would exit the chill tank either as post-chill negative or positive and is represented accordingly by the post-chill negative and post-chill positive outflows. The cross-contamination and the decontamination processes are represented by the "contaminated" and "decontaminated" flows. The effective chlorine and the cumulative count at post-chill are factors affecting the Y of the cross-contamination probability model. The effective chlorine is affected by turbidity and turbidity is affected by time, and these are already shown in the presentation. The Y is input into the cross-contamination probability model. The random contamination converter represents the stochastic nature of the probability model. On the other hand, the "Z" is affected by effective chlorine and cumulative count of post-chill positive broiler carcasses. The "Z" is a factor in the decontamination probability model which then goes into the random decontamination converter which calculates stochastically how many of the broiler carcasses are decontaminated in the chill tank. Thus, post chill negative and post-chill positive broiler carcasses are summed together as the "sum carcasses", and the post-chill prevalence is calculated as a percentage from the number of post-chill positive carcasses divided by the sum carcasses.

The model is able to represent the processes and components of the *Salmonella* contamination in the chill tank of the poultry processing plant, from the level of the poultry processing plant up to the level of the chill tank. Based on this, the model has good face validity in terms presented by Forester and Senge (1980). On the other hand,

Naylor and Finger (1967) described a model as having a "high face validity" if experts in the field agree that the model accurately represents the real system being modeled, and this requires a type of survey of the opinion of such field experts, which was beyond the scope of this study.

Sensitivity analysis

Sensitivity analysis for model behavior was tested by varying the parameter values of the model as suggested by Forester and Senge (1980). This was obtained by running a sensitivity analysis in the STELLA® program, using the sensitivity specs function tool and setting the pre-chill prevalence ranging from 0.1 to 0.9, with 9 runs each, and running the simulation at various settings of chlorine ranging from 0.06, the lowest recorded value in the study, up to 5 ppm which was the highest detectable by the probe. The final values of post-chill prevalence generated by the simulations were then copied to EXCEL file and graphed against the pre-chill prevalence observed for the seven trials.

The sensitivity analysis showed that the SD model of *Salmonella* contamination in the chill tank is able to predict the post-chill *Salmonella* contamination of broiler carcasses in the chill tank, in accordance with the relationships that are recorded in literature and also in the data collected in this research. Figure 5.4 shows the plot of the simulated post chill prevalence from the sensitivity analysis run in the STELLA® program. The predicted post chill prevalence of *Salmonella* at varying levels of pre-chill prevalence, and effective chlorine shows that as the level of effective chlorine increases, the level of *Salmonella* decreases; and as the pre-chill prevalence of *Salmonella* increases, the post-chill prevalence of *Salmonella* also increases. These relationships are

consistent with the reported relationships in literature for the pre-chill prevalence, post-chill prevalence, and chlorine. It was low when it was expected to be low, and high when expected to be high.

The predicted post-chill prevalence results based on the varying levels of chlorine are consistent with the existing knowledge. The FSIS requires that the chlorine level in the re-use water at the chill tank should be 1 to 5 ppm (USDA, 2010a). The highest predicted post-chill prevalence at a chlorine level less than 1 ppm shows that the disinfecting efficiency of chlorine is diminished below the level prescribed. Similarly, the highest activity of chlorine was seen as expected when the chlorine level is at the highest of 5 ppm (Figure 5.2).

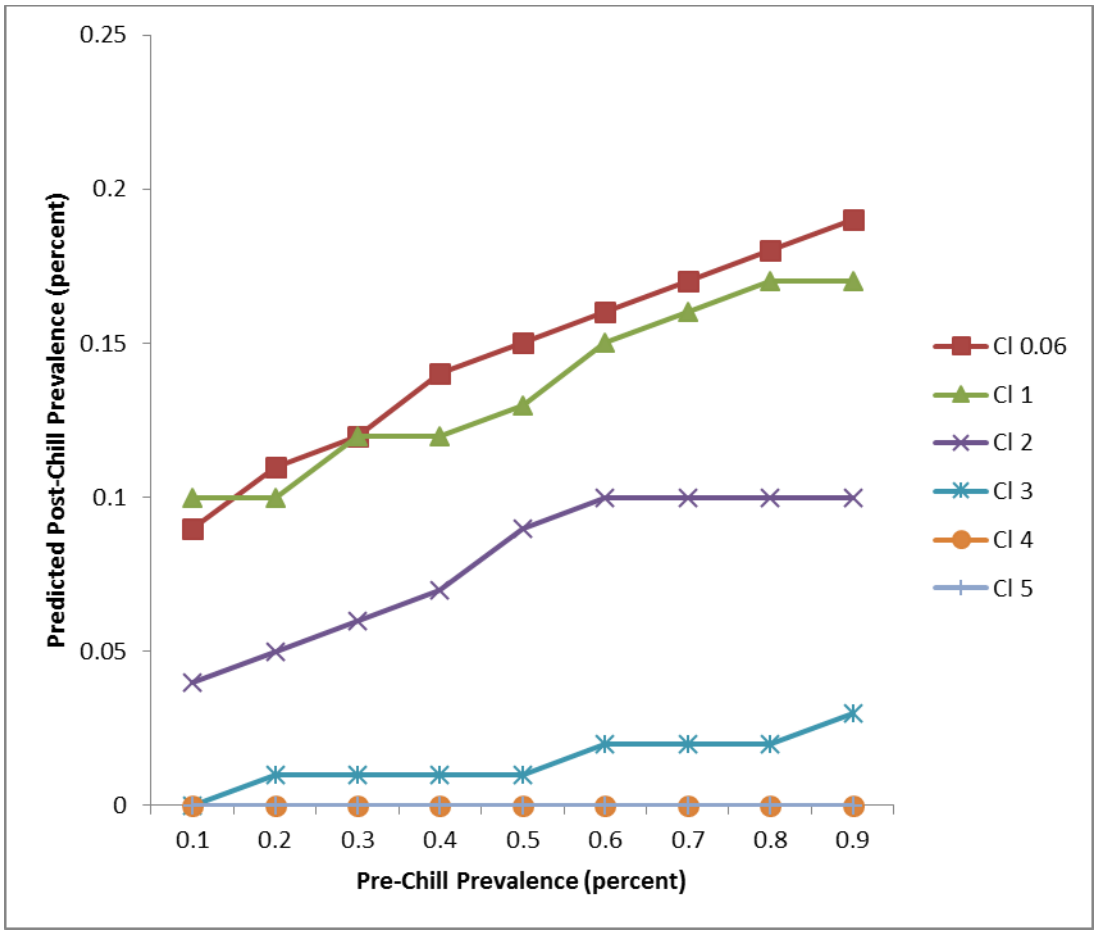

Figure 5.2 Predicted post-chill prevalence of *Salmonella* associated with varying levels of chlorine (Cl) in ppm and pre-chill prevalence

Tests of consistency for model behavior (Forester and Senge, 1980)

The next step is to compare the simulated post-chill prevalence with the actual observed post-chill prevalence. The STELLA program is used to run a simulation using the actual observed pre-chill prevalence and the mean chlorine level from the seven trials and input them in the model, and then record the resulting post-chill prevalence from the simulations. Figure 5.3 shows the scatter plot of the actual observed pre-chill prevalence versus the actual observed post-chill prevalence and the simulated post-chill prevalence.

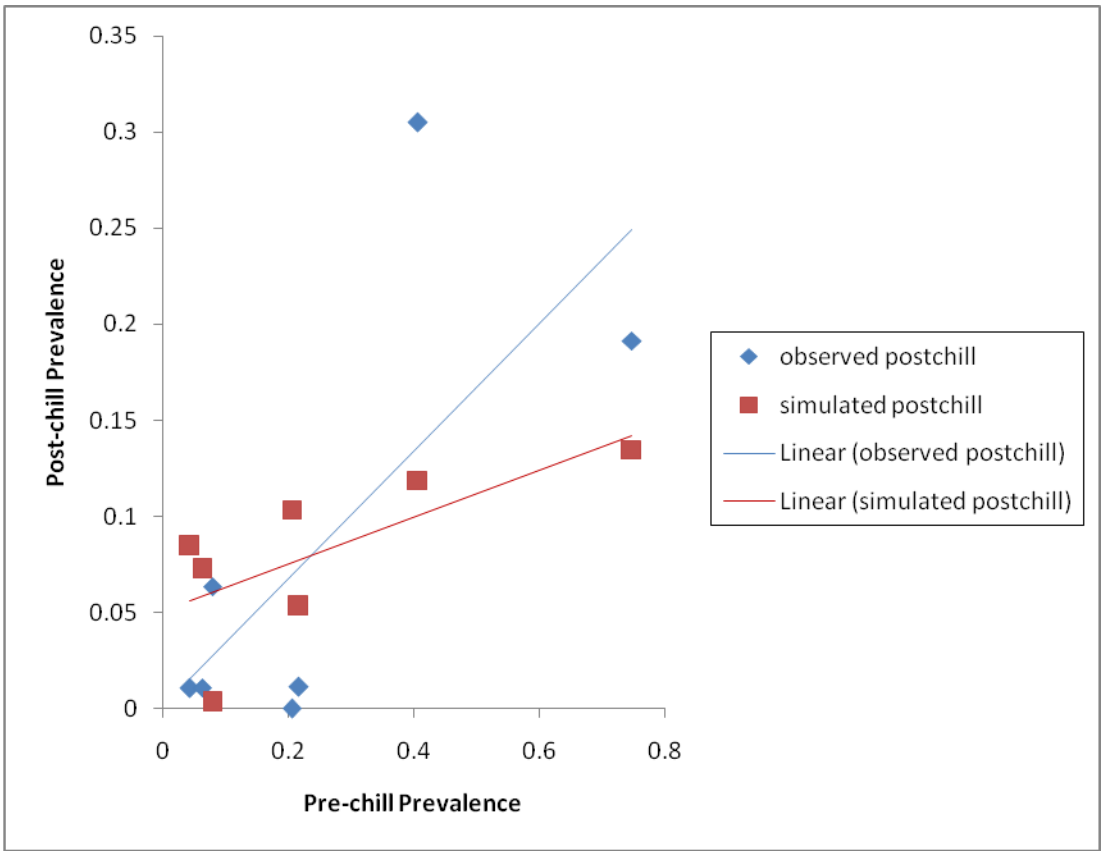

Figure 5.3 Scatter plot of observed vs simulated post-chill prevalence

It is interesting to compare the scatter plots of the observed and predicted post-chill prevalence vs. observed and predicted pre-chill prevalence. Although, the two trendlines are not exactly alike, they show a similar trend of increase which indicates that the SD model generated behavior fairly matches the behavior of the real system.

Assessment of validity

Tests of suitability of model structure

In addition to the above validity tests, an assessment was made on the validity of this system dynamics model of *Salmonella* contamination of broiler carcasses in the chill

tank of a processing plant using Forester and Senge's validation technique (Forrester and Senge, 1980).

The model structure is compatible with the real system since the components of the real system have been included in the structure of the model. The model does not contradict the knowledge about *Salmonella* contamination in the chill tank since the foundations of the model structure are based on existing knowledge of the real system. The most relevant structures of the real system namely, the flow of the carcasses in the processing plant (arrival, processing, speed of the line, chiller time), and the factors that affect *Salmonella* contamination in the chill tank (pre-chill *Salmonella* prevalence, pH, turbidity, temperature, hypochlorous acid) have been modeled. The sensitivity analysis shows that the equations in the model are robust and withstand extreme but possible values of the variables. The model passed the face validity test because the model structure looks like a simplified processing plant, with a zoom view into the chill tank. In addition, the feedback system in the model which is the post-chill cumulative count fits the characteristics of the real system. This feedback loop that is provided by the post-chill cumulative count on the system is probably one of the most important aspects of the model. Over time, the number of *Salmonella* carcasses that have passed through the chill tank has also left behind more bacterial units which impacts the number of post-chill positive broiler carcasses. The units used also correspond numerically to the ones that exist in the processing plant in the real life, particularly, time, pH, temperature, hypochlorous acid level, flock size, and number/sum of carcasses.

Test of utility and effectiveness for the model structure

The size of the SD model can be considered appropriate for the audience of the study. The model is simple enough and yet complex enough to capture the real system features. The complex part is more at the level of the chill tank, while the other parts of the model of the poultry processing plant is simpler. This is so that the audience can visualize the processing plant which is the bigger picture and also imagine the system of the *Salmonella* contamination at the microscopic level in the chill tank.

Tests of suitability for the model behavior

The sensitivity analysis showed that changing parameter values resulted in outcomes which were according to expectation of the real system for both the structure and the behavior aspect of the model. The predicted values of the various parameters were good for the model. Also, the model behaved in expected fashion under extreme values, which is similar to the way the real system would react, and this improves the confidence in the model (Saysel and Barlas, 2004).

Tests of utility and effectiveness for model behavior

It was interesting to note that the model has provided a new insight into the better understanding of the various relationships between the parameters in the chill tank that are contributing to *Salmonella* contamination. This further improves the validity of the model. Using actual data from the processing plant to develop the model has helped elucidate the relationships between the factors that affect *Salmonella* contamination of broiler carcasses in the chill tank. For example, the effect of turbidity on the disinfecting action of chlorine has been highlighted in the model.

In terms of this assessment, the model can be considered a valid model.

Conclusions

A growth probability model has been adapted to develop a cross contamination probability model and a decontamination probability model for predicting the contamination of a single broiler carcass at the level of the chill tank of a poultry processing plant. Furthermore, a system dynamics model of the *Salmonella* contamination in the chill tank of the processing plant has been developed based on actual data collected from a commercial poultry processing plant in Mississippi. The data was collected on seven sampling dates gathering thousands of real time data logs on the parameters. The system dynamics model helped elucidate the relationships between the various factors like pre-chill and post chill prevalence of *Salmonella*, and pH, temperature, time, turbidity, and effective chlorine of the chill tank water. Sensitivity analysis of the model shows that the model is able to effectively predict the post-chill contamination at various levels of chlorine and with varying pre-chill prevalence levels. The predicted post-chill prevalence of *Salmonella* is similar to the plot of pre-chill vs post-chill *Salmonella* prevalence from actual observed data which further shows the validity of the model. The model developed can be used to predict *Salmonella* contamination probability based on pre-chill prevalence, effective chlorine, pH, turbidity, flock size, and is a powerful tool for assessing interventions for preventing *Salmonella* contamination.

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CHAPTER VI

SUMMARY

The purpose of this research was to apply system dynamics simulation modeling and systems thinking to the problem of *Salmonella* contamination of broiler carcasses in the chill tank of the poultry processing plant. *Salmonella* contamination of broiler carcasses remains a problem in the 21st century. Indeed, after more than one hundred years of research, *Salmonella* still poses a challenge to human and animal health. It seemed like a new perspective was needed. The idea came as an inspiration after reading the book "Introduction to systems thinking" by Barry Richmond (Richmond and Peterson, 2000). Systems thinking as a heuristic offered a refreshing way of looking at the *Salmonella* problem. Where before *Salmonella* contamination was simply the presence or absence of *Salmonella*, and using chlorine and chilling to reduce *Salmonella* contamination at the chill tank, here was an opportunity to look at *Salmonella* contamination as not just a simple cause and effect, but as an actual system with various components interacting with one another. Thus was the birth of the initial idea of developing a system dynamics model of *Salmonella* contamination of broiler carcasses in the chill tank of the poultry processing plant.

However, getting to the actual development and validation of this model required several more important steps for it to be realized. First, the most sensitive method of carcass rinse that did not impact the *Salmonella* status of the samples had to be identified.

Chapter 2 or the "sampling methods" chapter investigated which sampling method was the most effective to use for studies using carcass rinses. Kappa agreement method was the statistical analysis that could help us determine which of three sampling methods was the best method, in that, it had minimal impact on the parameter being measured (*Salmonella* prevalence). Three methods were investigated namely, split carcass, consecutive rinse and adjacent pairs methods. Adjacent pairs method was found to be the preferable method based on consistent finding of moderate Kappa agreement between the adjacent pairs of carcass rinse samples on their *Salmonella* contamination status, and that there was no impact on the *Salmonella* status even after intervention (chilling) was introduced. For the split carcass rinse method, it was found that it proved to be too labor intensive and too costly. The consecutive rinse method showed inconsistent results in terms of Kappa agreement, and also there was a significant reduction in the prevalence of *Salmonella* between the consecutive rinse samples.

Another step was the literature based system dynamics (SD) model. In the absence of actual data, literature was searched for data to be used and which could be adapted to the SD model. In developing the literature based SD model of the *Salmonella* contamination of broiler carcasses in the chill tank of the processing plant, certain knowledge gaps were revealed. Based on its issuances and guidelines, the USDA believes that chlorine is important in controlling *Salmonella* contamination in the poultry processing plant, however, there is a lack of published data on how it actually works (USDA, 2006). Pre-chill and post-chill *Salmonella* prevalence had been reported but not for the individual broiler carcasses, and usually as a comparison after the intervention of chilling (Lillard, 1980; Tsai et al., 1992). A cross-contamination probability model had

been developed but it was based on experimentally inoculated drumsticks not whole carcasses. Yang et al. (2002) implied the effect of turbidity on chlorine as the age of the chiller water, but did not directly measure turbidity. Mead and Thomas (1973) measured turbidity however they related this to the number of carcasses that had already gone through the chiller rather than directly measuring it in time. The pH and temperature measurements and their impact on the dissociation of hypochlorous acid have been reported but not in relation to actual *Salmonella* contamination (White, 1999). Although it is known that poultry processing companies routinely monitor the pH level, the temperature, and the chlorine level of the water, these were not available or accessible for any analysis. Furthermore, there was no published data analysis on these factors in relation to *Salmonella* prevalence Thus, for the literature based model, meta-analysis of published reports was used to calculate the relationship of time, turbidity, chlorine levels, and *Salmonella* prevalence (Mead and Thomas, 1973; Yang et al., 2002). Using data from the literature, the literature- based SD model was developed and has potential as a tool for risk assessment or testing of interventions for reducing *Salmonella* contamination of poultry carcasses in the chill tank of the processing plant.

Using what was learned from developing the literature-based SD model, the next step was to try to fill in the knowledge gaps in the field. Thus, actual data was collected in field situation in a poultry processing plant using the adjacent pairs sampling method to collect the pre-chill and post-chill rinse samples, and the specially designed apparatus to measure and log the actual levels of pH, temperature, hypochlorous acid level, and turbidity every time a broiler carcass was sampled. The succeeding steps were made possible by the assembly of the portable apparatus that had the probes that could measure

the pH, actual data collection, data logging, sample collection and laboratory testing. Through this apparatus, it was possible to collect the immense amount of data on the factors over time in the chill tank and could be correlated with each other and with the *Salmonella* pre-chill and post-chill prevalence data as well.

In Chapter 4, the relationships between pH, time, temperature, pre-chill prevalence, turbidity, and hypochlorous acid were analyzed. One of the main findings was that the turbidity of the chiller water was found to increase over time. This is quite logical since as time goes on; more carcasses go through the chiller thereby increasing the organic matter level therein which in turn makes the water more turbid. On the other hand, the findings showed a negative relationship between free available chlorine (HOCl) and turbidity. The level of free available chlorine HOCl decreased as the turbidity of the chiller water increased. This is because higher turbidity levels indicate more organic matter in the chiller water which then binds the chlorine leaving less free available chlorine. This highlighted an important aspect of chlorine's bactericidal activity. Not only is chlorine's bactericidal activity affected by the pH, but also by the turbidity of the chiller water. As for the temperature of the chiller water, it was found to increase with time. This is important because the temperature of the chiller water has to be kept at 4 degrees Celsius or below in order to prohibit the growth of *Salmonella*. The pre-chill prevalence did not have a significant relationship with any of the parameters. As time increased, the pH also increased. However, this relationship was not factored in the data-based SD model, instead, the pH was used as a variable that could be set with a specific set of values, which is more typical of the poultry processing plant scenario. The

processing plants maintain the pH level of their chill tank and do not allow it to fluctuate over time.

In Chapter 5, analyzing the results of these field observations and sample collections, we found that the literature based model had to be modified. Unlike the literature based model, wherein the regression model for the cross-contamination probability model used three parameters – age of chiller water, pre-chill incidence and chlorine, it was quite different for the data-based SD model. This time, the pre-chill incidence was still important but as a variable that determined the flow of the broiler carcass- pre-chill positive or pre-chill negative rather than as a function of Y. In the Data-Based SD Model, Y was a function of effective chlorine and post-chill incidence and their interaction. On the other hand, effective chlorine was a function of turbidity and free chlorine. Turbidity was a function of time, and free chlorine was a function of percent hypochlorous acid and initial chlorine. Temperature was a function of minute (time). Temperature was a factor affecting the hypochlorous acid dissociation constant. On the other side of the model, the decontamination side, Z was a function of effective chlorine, post-chill incidence and their interaction. Thus, using the actual data, the literature-based data was improved and developed into the data-based model.

Epidemiologically, the concept of contamination is based on the concept of the disease triad where the three points of interest are the pathogen, the host and the environment. In the chill tank of the poultry processing plant, and in developing this data-based SD model, the focus is on *Salmonella* as the pathogen, the broiler carcass as the host and the chill tank as the environment. The pH, the temperature, the turbidity, the chlorine level of the chiller water may be considered as part of the environment in this

system. Changes in the environment have an effect on *Salmonella*, and on whether or not it is able to contaminate the broiler carcass in the chill tank or the changes in the environment may cause the decontamination or removal of *Salmonella*.

In the second half of Chapter 5, the data-based SD model was validated in order to prove its rationality and sensibility, and to support or promote its acceptability. This process of development and formulation of the SD model described here made it possible to really zoom in on the chill tank, the broiler carcass, and the dynamic system in the chill tank. The model developed is both a small picture and a big picture of *Salmonella* contamination: the process or the phenomenon. It is a small picture considering that it offers the microscopic view of the system such as the pH, the bacterial contamination, and dissociation of hypochlorous acid, etc. It is also at the same time a big picture of *Salmonella* contamination since the entire processing plant is modeled in a summarized way.

The STELLA® Data-Based SD model had to go through a lot of revisions. As we knew more from our data, about the relationships of the factors, we had to change the connections in our model. Thus the resulting model elucidates a lot about what is really happening in the chill tank, and how each factor contributes to the next and to the next step. There were times during the stepwise model fitting that the model was not doing what it was supposed to be doing, and it was during those times that the various levels of the model had to be reviewed, recalculated and duly corrected. An important lesson in all these was that the data-based SD model is a tool for understanding the system better. This data-based system dynamic model of *Salmonella* contamination can be a powerful tool for the poultry industry for risk assessment, risk reduction and for testing

intervention measures. Our results show that it is indeed possible to recognize *Salmonella* contamination of broiler carcasses in the chill tank of a processing plant as a complex system consisting of the various factors that are interrelated dynamically and with a feedback mechanism.

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APPENDIX A

COMPLETE LIST OF FORMULAS FOR THE LITERATURE-BASED SD MODEL OF *SALMONELLA* CONTAMINATION OF BROILER CARCASSES IN THE CHILL TANK OF A POULTRY PROCESSING PLANT

Arrival(t) = Arrival(t - dt) + (- Birds_per_Minute) * dt

INIT Arrival = Flock_Size

OUTFLOWS:

Birds_per_Minute = Flock_Size/Chiller_Time

Post_Chill_Negative(t) = Post_Chill_Negative(t - dt) + (Stays_Negative +

Decontaminated) * dt

INIT Post_Chill_Negative = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Stays_Negative = CONVEYOR OUTFLOW

Decontaminated = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Decontam

NO-LEAK ZONE = 0

Post_Chill_Positive(t) = Post_Chill_Positive(t - dt) + (Contaminated + Stays_Positive) *

dt

INIT Post_Chill_Positive = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Contaminated = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Contam

NO-LEAK ZONE = 0

Stays_Positive = CONVEYOR OUTFLOW

PreChill_Negatives(t) = PreChill_Negatives(t - dt) + (Split_Negatives - Stays_Negative - Contaminated)*dt

INIT PreChill_Negatives = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Split_Negatives = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Status

NO-LEAK ZONE = 1

OUTFLOWS:

Stays_Negative = CONVEYOR OUTFLOW

Contaminated = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Contam

NO-LEAK ZONE = 0

PreChill_Positives(t) = PreChill_Positives(t - dt) + (Split_Positives - Stays_Positive - Decontaminated) * dt

INIT PreChill_Positives = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Split_Positives = CONVEYOR OUTFLOW

OUTFLOWS:

Stays_Positive = CONVEYOR OUTFLOW

Decontaminated = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Decontam

NO-LEAK ZONE = 0

Processing(t) = Processing(t - dt) + (Birds_per_Minute - Bird_Entry) * dt

INIT Processing = 0

TRANSIT TIME = varies

INFLOW LIMIT = 200

CAPACITY = INF

INFLOWS:

Birds_per_Minute = Flock_Size/Chiller_Time

OUTFLOWS:

Bird_Entry = CONVEYOR OUTFLOW

TRANSIT TIME = Chiller_Time

Status_Split(t) = Status_Split(t - dt) + (Bird_Entry - Split_Positives - Split_Negatives) *

dt

INIT Status_Split = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Bird_Entry = CONVEYOR OUTFLOW

TRANSIT TIME = Chiller_Time

OUTFLOWS:

Split_Positives = CONVEYOR OUTFLOW

Split_Negatives = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Status

NO-LEAK ZONE = 1

Chiller_Time = 100

Flock_Size = 100000

Flow_Rate = 8.0

Free_Chlorine = Initial_Chlorine*PCT_HOCL/100

H_Conc = 10^-pH

Initial_Chlorine = 50

Ka = 10^-((3000/(Temp+273))-10.0686+(0.0253*(Temp+273)))

PCT_HOCL = (1/(1+(Ka/H_Conc)))*100

pH = 7

Post_Chill_Prevalence = IF Sum_Carcasses=0 then 0 else

(Post_Chill_Positive/Sum_Carcasses)*100

PreChill_Prevalence = 25

Random_Contam = IF (RANDOM(0, 100, 20))<= X_Contam__Prob THEN 1 ELSE 0

Random_Decontam = IF (RANDOM(0, 100, 10))<= Decontam__Probability THEN 1 ELSE 0

Random_Status = IF (RANDOM(0, 100, 20))<= PreChill_Prevalence THEN 0 ELSE 1

Sum_Carcasses = Post_Chill_Negative+Post_Chill_Positive

Temp = 2

Turbidity = if Sum_Carcasses <=60000 then (17.71532+ 0.00091737*(Sum_Carcasses) -1.84695*(Flow_Rate)) else 40

X_Contam__Prob = ((1/(1+EXP(-Y))))*100

Y = -3.5099-(0.0336*Free_Chlorine)+(0.0583*PreChill_Prevalence)+(0.8866*Age_of__Chiller_Water)

Z = -1.3018-(0.0319*Free_Chlorine)+(0.8767*Age_of__Chiller_Water)

Age_of__Chiller_Water = GRAPH(Turbidity)

(6.00, -1.00), (23.0, 0.00), (40.0, 1.00)

Decontam__Probability = GRAPH(((1/(1+EXP(-Z))))*100)

(40.0, 1.00), (95.0, 50.0), (150, 99.0)

APPENDIX B

COMPLETE LIST OF FORMULAS IN THE DATA-BASED SD MODEL OF *SALMONELLA* CONTAMINATION OF BROILER CARCASSES IN THE CHILL TANK OF A POULTRY PROCESSING PLANT

Arrival(t) = Arrival(t - dt) + (- Shackle_Rate) * dt

INIT Arrival = Flock_Size

OUTFLOWS:

Shackle_Rate = Birds_per_Minute

Post_Chill_Negative(t) = Post_Chill_Negative(t - dt) + (Stays_Negative +

Decontaminated) * dt

INIT Post_Chill_Negative = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Stays_Negative = CONVEYOR OUTFLOW

Decontaminated = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Decontam

NO-LEAK ZONE = 0

Post_Chill__Positive(t) = Post_Chill__Positive(t - dt) + (Stay_Pos_Exit +

Cross_Contam__Exit) * dt

INIT Post_Chill__Positive = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Stay_Pos_Exit = CONVEYOR OUTFLOW

Cross_Contam__Exit = CONVEYOR OUTFLOW

Pre-chill_Negatives(t) = Pre-chill_Negatives(t - dt) + (Split_Negatives - Stays_Negative - contaminated) * dt

INIT Pre-chill_Negatives = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Split_Negatives = LEAKAGE OUTFLOW

LEAKAGE FRACTION = 1-(Pre-chill_Prevalence)

NO-LEAK ZONE = 1

OUTFLOWS:

Stays_Negative = CONVEYOR OUTFLOW

contaminated = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Contam

NO-LEAK ZONE = 0

Pre-chill_Neg_Sum(t) = Pre-chill_Neg_Sum(t - dt) + (Pre-chill_Neg_Flow) * dt

INIT Pre-chill_Neg_Sum = 0

INFLOWS:

Pre-chill_Neg_Flow = Pre-chill_Neg_Count

Pre-chill_Positives(t) = Pre-chill_Positives(t - dt) + (Split_Positives - Decontaminated - stays_positive) * dt

INIT Pre-chill_Positives = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Split_Positives = CONVEYOR OUTFLOW

OUTFLOWS:

Decontaminated = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Decontam

NO-LEAK ZONE = 0

stays_positive = CONVEYOR OUTFLOW

pre-chill_pos_sum(t) = pre-chill_pos_sum(t - dt) + (pre-chill_pos_flow) * dt

INIT pre-chill_pos_sum = 0

INFLOWS:

pre-chill_pos_flow = pre-chill_pos_count

Processing(t) = Processing(t - dt) + (Shackle_Rate - Bird_Entry) * dt

INIT Processing = 0

TRANSIT TIME = varies

INFLOW LIMIT = 200

CAPACITY = INF

INFLOWS:

Shackle_Rate = Birds_per_Minute

OUTFLOWS:

Bird_Entry = CONVEYOR OUTFLOW

TRANSIT TIME = Chiller_Time

Status_Split (t) = Status_Split(t - dt) + (Bird_Entry - Split_Positives - Split_Negatives)

*dt

INIT Status_Split = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

Bird_Entry = CONVEYOR OUTFLOW

TRANSIT TIME = Chiller_Time

OUTFLOWS:

Split_Positives = CONVEYOR OUTFLOW

Split_Negatives = LEAKAGE OUTFLOW

LEAKAGE FRACTION = 1-(Pre-chill_Prevalence)

NO-LEAK ZONE = 1

stay_positive_total(t) = stay_positive_total(t - dt) + (stays_positive - Stay_Pos_Exit) * dt

INIT stay_positive_total = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

stays_positive = CONVEYOR OUTFLOW

OUTFLOWS:

Stay_Pos_Exit = CONVEYOR OUTFLOW

Stay_Pos_Sum(t) = Stay_Pos_Sum(t - dt) + (Stay_Pos_Flow) * dt

INIT Stay_Pos_Sum = 0

INFLOWS:

Stay_Pos_Flow = Stay_Pos_Count

X_Contam_Sum(t) = X_Contam_Sum(t - dt) + (X_Contam_Flow) * dt

INIT X_Contam_Sum = 0

INFLOWS:

X_Contam_Flow = X_Contam_Count

X_Contam_Total(t) = X_Contam_Total(t - dt) + (contaminated - Cross_Contam__Exit) *

dt

INIT X_Contam_Total = 0

TRANSIT TIME = 1

INFLOW LIMIT = INF

CAPACITY = INF

INFLOWS:

contaminated = LEAKAGE OUTFLOW

LEAKAGE FRACTION = Random_Contam

NO-LEAK ZONE = 0

OUTFLOWS:

Cross_Contam__Exit = CONVEYOR OUTFLOW

Birds_per_Minute = Flock_Size/Chiller_Time

CCUMCNT = 444 * (Post_Chill__Positive/Flock_Size)

Chiller_Time = 100

cum_count_for_positive = 155*Post_Chill__Positive/Flock_Size

Decontam__Probability = 100-((1/(1+EXP(-z))))*100

Effective__Chlorine = Free_Chlorine+((-0.00445)* (Turbidity))

Flock_Size = 20000

Free_Chlorine = Initial_Chlorine*PCT_HOCL/100

H_Conc = 10^-pH

Initial_Chlorine = 3

Ka = 10^-((3000/(Temp+273))-10.0686+(0.0253*(Temp+273)))

minute = Sum_Carcasses/Birds_per_Minute

PCT_HOCL = (1/(1+(Ka/H_Conc)))*100

pH = 6

Post_Chill_Prevalence = IF Sum_Carcasses=0 then 0 else

(Post_Chill__Positive/Sum_Carcasses)

Pre-chill_Neg_Count = Pre-chill_Negatives

pre-chill_pos_count = Pre-chill_Positives

Pre-chill_Prevalence = .1

Pre-chill_Prev_Pct = .01

Random_Contam = IF (RANDOM(0, 100, 20))<= X_Contam__Prob THEN 1 ELSE 0

Random_Decontam = IF (RANDOM(0, 100, 20))<= Decontam__Probability THEN 1

ELSE 0

Stay_Pos_Count = stay_positive_total

Sum_Carcasses = Post_Chill_Negative+Post_Chill__Positive

Temp = 6.1616+(0.005390*minute)

Turbidity = -1.8573+(0.19 * minute)

X_Contam_Count = X_Contam_Total

X_Contam__Prob = ((1/(1+EXP(-Y))))*100

Y = 2.4021-(3.7399*Effective__Chlorine) -(0.2299* CCUMCNT) +

(0.1536*Effective__Chlorine*CCUMCNT)

z = 0.4893-(1.3639*Effective__Chlorine) - (0.04195*cum_count_for_positive) +(

0.02809* Effective__Chlorine*cum_count_for_positive)