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EFFECTS OF MONENSIN AND SODIUM CHLORATE ON *SALMONELLA* INFANTIS COLONIZATION IN BROILERS

By

Geetha S. Kumar

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ABSTRACT

Effects of monensin and sodium chlorate on *Salmonella* Infantis colonization in broilers

By

Geetha S. Kumar

Two hundred and ten *Salmonella*-free broilers were raised on feed with or without monensin. All chicks were orally infected on day 3 with *S.* Infantis (10^8 CFU / bird). Caecal colonization by *S.* Infantis was evaluated on days 7, 10, 14, 21, 28, 35, and 42. Both groups had similar caecal concentrations of *S.* Infantis, indicating that monensin was not an effective means of reducing *S.* Infantis colonization. Next, 150 *Salmonella*-free broilers were divided into four groups, three of which received a commercial sodium chlorate solution days 10, 20, and 38 for four consecutive days with the last group given no sodium chlorate treatment. All chicks were orally infected on day 3 with *S.* Infantis (10^8 CFU / bird). Caecal colonization and liver invasion by *S.* Infantis were assessed on days 7, 10, 14, 17, 20, 24, 27, 30, 38, and 42 with fecal swab samples also analyzed on days 11, 12, 13, 15, 16, 18, 19, 21, 22, 23, 25, 26, 28, 29, 39, 40, and 41. Birds receiving sodium chlorate on days 10 and 20 yielded significantly lower caecal populations of *S.* Infantis up to 7 days after treatment ($P<0.05$) compared to the untreated control group. No significant reduction of *Salmonella* was observed in birds treated with sodium chlorate on day 38 and in the liver invasion. However fecal shedding of *S.* Infantis decreased 2.0 to 3.5 logs three to four days after treatment with sodium chlorate on days 10 and 20.

Lovingly dedicated to my Guruji, Sri Sri Ravi Sankar, parents, Radha & Sanal,

brother, Madhu, and dearest son, Akshay

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CHAPTER 1

INTRODUCTION

Salmonella, one of the leading causes of foodborne illness in the United States, is responsible for an estimated 1.4 million cases and 553 deaths annually with associated costs of $2.4 billion (Mead et al., 1999). According to the Center for Science in the Public Interest (CSPI, 2001), poultry products rank among the top five food vehicles responsible for outbreaks of foodborne illness. The other four vehicles listed were seafood, eggs, produce and beef. The United States Department of Agriculture - Food Safety and Inspection Service (USDA-FSIS) issued the Pathogen Reduction / Hazard Analysis and Critical Control Point program final rule in July of 1996 to reduce the incidence of foodborne pathogens in raw meat and poultry products. According to this program, *Salmonella* performance standards, which are product specific, were determined from nationwide microbial baseline data. Based on performance standards, the maximum allowable *Salmonella* contamination rate for broilers was fixed at 20%. After implementation of HACCP, more than 80% of samples met the *Salmonella* performance standards from 1998 to 2000 (Rose et al., 2002) with FSIS expecting this trend to continue so that the targeted incidence rate of 6.8 cases of salmonellosis / 100,000 persons is achieved by the year 2010.

Efforts to meet the 2010 projection for salmonellosis should be aimed at reducing the prevalence of *Salmonella* in poultry. Broilers are widely recognized as reservoirs for *Salmonella*, which colonizes and proliferates in the gastrointestinal tract of these birds. The primary source of *Salmonella* in finished poultry products is the infected gastrointestinal tract of birds. Thus, control measures aimed at reducing *Salmonella* colonization in the gastrointestinal tracts of birds will be very rewarding to the poultry industry.

One of the age-old methods to reduce *Salmonella* colonization in birds is the use of antibiotic feed additives. Antibiotics are added at sub-therapeutic concentrations to animal feed to prevent diseases as well as to improve growth rate and weight gain. Antibiotics such as chlortetracycline, neomycin and oxytetracycline have proven to be effective against *Salmonella* and have been commonly used as poultry feed additives (Quarles et al., 1977; Williams, 1985). The practice of feeding animals sub-therapeutic levels of antibiotics often leads to selective pressures that result in the emergence of new, antibiotic-resistant strains of *Salmonella* and other pathogens in the food chain (Dupont and Steele, 1987; Holmberg, 1987; D'Aoust 1989). Foodborne infections are becoming increasingly difficult to treat with the emergence of multi-antibiotic resistant bacteria such as *Salmonella* Typhimurium DT 104 in the food chain (Wall et al., 1994).

Given the increasing problem of antibiotic resistance, the poultry industry has developed alternative measures such as competitive exclusion to control *Salmonella*. Competitive exclusion cultures, obtained from mucosal wall scrapings of pathogen-free adult birds, have reportedly reduced *Salmonella* colonization (Blankenship et al., 1993; Bailey et al., 2000). However, some commercially available competitive exclusion products have proven to be ineffective in protecting chicks against *Salmonella* infections (Stavric et al., 1992). A new approach gaining popularity is the feeding of sodium chlorate to food-producing animals to reduce *Salmonella* colonization. Bactericidal activity of sodium chlorate has been demonstrated against several important foodborne pathogens in the Family *Enterobacteriaceae* including *Salmonella* and *Escherichia coli* O157:H7 (Anderson et al., 2000). Both of these organisms possess respiratory nitrate reductase activity which can bring about the anaerobic reduction of nitrate to nitrite as well as intracellular reduction of chlorate to chlorite. The resulting chlorite is cytotoxic and is lethal to those bacteria possessing the nitrate reductase enzyme which includes *Salmonella*. In one such study, *Salmonella* populations in the caeca of pigs decreased by two logs following oral administration of a sodium chlorate solution (Anderson et al., 2001).

The goal of this study was to investigate two treatments for broilers, which may help reduce colonization by *Salmonella*, and thereby reduce the incidence of *Salmonella* on broiler carcasses. For this purpose, effects of the ionophore antibiotic feed additive monesin and sodium chlorate on *Salmonella* colonization and fecal shedding in broilers were investigated.

CHAPTER 2

LITERATURE REVIEW

2.1. FOOD SAFETY

Food safety issues are becoming a major concern worldwide due to the increasing incidence of foodborne disease. In the United States, an estimated 76 million cases of foodborne illness occur annually, resulting in 325,000 hospitalizations and 5,000 deaths costing approximately 9.2 billion dollars (Mead, et al., 1999). Public health authorities believe that most cases of foodborne illness are unreported since the symptoms are often mild and do not require medical attention. Hence, it is very difficult to obtain accurate data on the true incidence of foodborne illness.

Norwalk virus now accounts for most cases of foodborne illness (23,000,000) followed by the bacterial pathogens *Campylobacter* (2,453,926) and *Salmonella* (1,412,498) (Mead, et al., 1999). Since 1985, several bacterial pathogens including *Listeria monocytogenes*, *Escherichia coli* O157:H7 and antibiotic-resistant strains of *Salmonella* have emerged as serious threats to the food supply. According to the Center for Science in the Public Interest (CSPI), poultry ranks fifth among the top five single-food vehicles in outbreaks of foodborne illness reported from 1990 to 2001 (CSPI, 2001). The other vehicles listed in the order of rank were seafood, eggs, produce and beef. Hence, the microbiological quality of raw poultry and poultry products has become a major public health issue.

The risks associated with consumption of contaminated food continue to change. The current rise in foodborne illness may be due to a wide range of factors including rapid urbanization in developing countries, movement of people as well as live animals and food products across borders, changes in food handling practices, changes in raw material sources, failure to follow standard hygienic procedures, development of ready-to-eat foods with extended refrigerated shelf life and emergence of new pathogens as well as antibiotic resistance and mutations among existing pathogens. In addition, changes in demographics are playing a major role with the very young and the aged as well as pregnant and immunocompromised individuals being more susceptible to foodborne illness than the general population (Gerba et al., 1996). Control of bacterial pathogens has been further complicated by new modes of bacterial transmission and resistance to food processing/preservation strategies. While most foodborne illnesses produce temporary symptoms, salmonellosis, one of the leading bacterial foodborne illness, can lead to more serious complications such as reactive arthritis or Reiter's syndrome - a long-term chronic illness characterized by joint pain, eye irritation and painful urination (Frenzen et al., 1999).

In the United States, six federal agencies have primary responsibility for food safety: two agencies under the Department of Health and Human Services – the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC); three agencies under the USDA – the Food Safety and Inspection Service (FSIS), the Agricultural Research Service (ARS) and the

Cooperative State Research, Education and Extension Service (CSREES); and the Environmental Protection Agency (EPA). While the primary role of the CDC in food safety is to identify and track foodborne disease outbreaks through FoodNet and PulseNet, the CDC also supports disease surveillance, research, preventative efforts and training related to foodborne diseases. USDA-FSIS is the agency responsible for ensuring the safety, wholesomeness and accurate labeling of meat and poultry products. According to the Federal Meat Inspection Act and Poultry Products Act, the sale or transportation of adulterated or misbranded meat and poultry products is prohibited. The Food, Drug and Cosmetic Act of 1938 states that a food may be adulterated and therefore unfit for human consumption if it contains harmful substances (e.g. pathogenic bacteria or microbial toxins). FSIS is primarily concerned with the presence of bacterial pathogens and their toxins in foods, as these are responsible for moderate to severe illness and/or death.

FSIS issued the Pathogen Reduction / Hazard Analysis and Critical Control Points (PR / HACCP) rule on July 25, 1996 (Federal register, 1996) to enhance the safety of meat and poultry products. According to this rule, all federally regulated livestock, poultry slaughter and processing facilities were required to develop HACCP programs. These facilities were also required to meet pathogen reduction performance standards for *Salmonella* (fixed at 20% according to baseline data collected by the USDA) and initiate microbial testing programs to ensure compliance with the targets. FSIS is also testing for *Salmonella* on raw meat and poultry products to determine whether these performance standards are being met. FSIS reported a substantial decline in the incidence of *Salmonella* from the baseline performance standards from 1998 to 2000 after the implementation of HACCP programs (Rose et al., 2002). Only 10.8% of broiler carcasses tested at the processing plants were positive for *Salmonella* with this reduction attributed to the implementation of HACCP (FSIS-USDA, 2002). Along with this reduction, CDC reports a 15% decline in the incidence rate of Salmonella infections in humans during 1996-2001 (MMWR, 2002). Despite all these reports of reduction, *Salmonella* still remains the leading bacterial foodborne pathogen with an incidence rate of 13.8 cases/100,000 people (MMWR, 2002). Most recently, the national health objectives target for Salmonella was fixed at 6.8 cases per 100,000 persons and it is expected to be achieved by the year 2010 (MMWR, 2002). Since the primary source of *Salmonella* contamination in poultry processing environments is the gastrointestinal tracts of incoming birds (Bailey, 1993), efforts aimed at reducing *Salmonella* colonization within the intestinal tracts should prove very rewarding for the poultry industry.

2.2. FOODBORNE BACTERIAL PATHOGENS IN POULTRY

The most important bacterial pathogens associated with poultry and poultry products include *Salmonella*, *Campylobacter* and *Listeria monocytogenes* with the first two organisms being the two primary causes of bacterial foodborne gastroenteritis (Todd, 1980; Archer and Kvenberg, 1985; Bryan and Doyle, 1995; Zhao et al., 2001). Of the 76 million estimated cases of foodborne illnesses, nearly 2.4 million cases are caused by *Campylobacter* species with an additional 1.4 million cases caused by non-typhoidal *Salmonella* (Mead et al., 1999). *Salmonella* usually causes foodborne outbreaks involving large numbers of people. Campylobacter, on the other hand causes more numbers of outbreaks with smaller numbers of people involved in each outbreak.

Regular production of poultry meat became possible due to the development of fast-growing broiler chickens, selective breeding programs, vaccines and antibiotics to control diseases, improved nutrition, and automation and integration of different production units in the poultry industry. The demand for chicken products, as well as per capita consumption of poultry meat, has risen dramatically with per capita consumption having increased from 35 lbs in 1970 to 70 lbs in 1997 (USDA, 1998).

With increased consumption of poultry, poultry-associated foodborne outbreaks have also increased. CDC reported that foodborne outbreaks due to contaminated chicken and turkey accounted for 1.5%, 1.9% and 2.4% of all foodborne outbreaks in the United States during 1995, 1996 and 1997, respectively (CDC, 2000), with *Salmonella* and *Campylobacter* being the most important bacterial pathogens associated with poultry and poultry products worldwide (Bryan and Doyle, 1995).

2.3. SALMONELLOSIS

Salmonellosis is of primary concern in the United States and can be acquired by ingesting undercooked or recontaminated poultry or by cross-contamination of other foods through improper handling of raw poultry (Bryan and Doyle, 1995). It is estimated that the presence of *Salmonella* in poultry products results in an estimated 409,624 cases of human illnesses, 160 deaths and associated costs of $ 6.96 million, all of which are extremely detrimental to the poultry industry (Mead et al., 1999; CSPI, 2001).

Salmonella, first recognized as a foodborne pathogen in the 1880's (Marth, 1969), has now emerged as a major cause of foodborne gastroenteritis worldwide. Salmonellosis can be caused by any one of over 2000 serovars of *Salmonella*. The serovars involved in salmonellosis can vary geographically but most frequently include *S.* Enteritidis, *S.* Typhimurium, *S.* Agona, *S.* Newport, *S.* Heidelberg, *S.* Infantis, *S.* Saintpaul, *S.* Hadar and *S.* Weltevreden. While some of these serovars maintain their dominant role in foodborne infections, others emerge and decrease over time (Uyttendaele et al., 1998).

Salmonella, one of the most frequently reported foodborne pathogens in the United States and other developed countries, is a common contaminant of fresh poultry (Bean and Griffin, 1990; FSIS-USDA, 2002). Active surveillance for salmonellosis started in 1955 with annual reports of laboratory isolations from humans in the United States continuing to increase as a result of FoodNet. However, most cases of salmonellosis are not reported due to mild symptoms that often go unnoticed.

Typical clinical syndromes produced by non-typhoid *Salmonella* include gastroenteritis, septicaemia and focal infections of the eye, joints, lungs, kidneys, heart and brain that may follow untreated or prolonged septicaemia (D'Aoust, 1994). Gastroenteritis is characterized by abdominal pain, diarrhea and fever and chills with nausea. Vomiting and headache occur less frequently. The onset time for salmonellosis usually ranges from 7 to 72 hours with clinical signs most often appearing 12 to 36 h after exposure. The duration of diarrhea is usually three to five days but can persist for up to two weeks. In about half of all cases, the organism will be shed for two to four weeks with fecal shedding normally ceasing after two to three months. *Salmonella* infections can also lead to secondary complications such as reactive arthritis or Reiter's syndrome - a long-term chronic illness characterized by joint pain, eye irritation, and painful urination. The most severe infections typically occur in infants, the elderly and immunocompromised individuals. Salmonellosis has been steadily increasing as a public health problem over the last 40 years in the United States. Reports estimate the number of all *Salmonella* food borne illness cases as 1.4 million annually with 553 deaths and associated costs of $2.4 billion (ERS, USDA, 2002).

At least 25 poultry-related outbreaks of salmonellosis have been documented since 1982 (Table 1). One such outbreak associated with consumption of poultry giblets occurred at a Maine restaurant (MMWR, 1984). One hundred and twelve culture-confirmed cases involving *Salmonella enteritidis* serotype Enteritidis were identified after diners consumed giblet gravy prepared from refrigerated uncooked giblets over the Thanksgiving weekend.

Table 1: Food borne illness associated with poultry and poultry products

Year	Suspected vehicle	Serovar	No: of cases	Location	Reference
1982	poultry giblets	*S.* Enteritidis	112	restaurant, Maine	MMWR, 1984
1983	chicken livers	*S.* Heidelberg	7	restaurant, Maine	MMWR, 1984
1985	turkey salad	*S.* Enteritidis	351	school, Georgia	MMWR, 1985
1994	ice-cream	*S.* Enteritidis	80	Minnesota	MMWR, 1994
1995	baked eggs	*S.* Enteritidis	70	nursing home, Indiana	MMWR, 1996
1995	stuffing	*S.* Enteritidis	7	private home, Nevada	MMWR, 1996
1995/1996	handling live chicks	*S.*Montevideo	23	Idaho/ Washington	MMWR, 1997
1996	handling live poultry	*S.*Montevideo	16	Oregon	MMWR, 1997
1997	chicken salad / potato salad	*S.* Enteritidis	48	restaurant, Ohio	CDC, 1997
1997	chicken	*S.*Hadar	39	restaurant, Virginia	CDC, 1997
1997	chicken	*S.*Heidelberg	15	private home, California	CDC, 1997

1998	turkey & stuffing	*S.* Heidelberg	25	church / temple, PA	CDC, 1998
1998	chicken pot pie	*S.*Muenchen	2	restaurant, Maine	CDC, 1998
1998	chicken tandori	*Salmonella* spp.	43	Illinois	CDC, 1998
1998	chicken kiev	*S.*Typhimurium	15	private home, Minnesota	CDC, 1998
1998	chicken	*Salmonella*	2	private home, Florida	CDC, 1998
1999	chicken	*S.* Braenderup	7	private home, California	CDC, 1999
1999	ice-cream	*S.* Enteritidis	256	fair/ festival, Utah	CDC, 1999
1999	chicken	*S.* Give	19	Wisconsin	CDC, 1999
1999	baked chicken	*S.* Hadar	104	Pennsylvania	CDC, 1999
1999	chicken	*S.*Javiana	7	restaurant, Texas	CDC, 1999
1999	turkey & stuffing	*S.* Saintpaul	5	restaurant, MA	CDC, 1999
1999	roasted turkey	*S.* Stanley	34	daycare center, NY	CDC, 1999
1999	handling live poultry	*S.* Typhimurium	40	Missouri	MMWR, 2000
1999	handling chicks & ducklings	*S.* Infantis	21	Michigan	MMWR, 2000

A similar outbreak traced to liver pate occurred in Maine in October of 1983 at another restaurant belonging to the same restaurant chain as the previous outbreak (MMWR, 1984). The implicated liver pate was prepared from frozen chicken livers that had been defrosted over a period of 4 days in a refrigerator before use. Positive cultures from seven individuals who dined at the restaurant confirmed the causative agent as *Salmonella* Heidelberg.

In 1985, a turkey-associated salmonellosis outbreak was identified among children and staff at a Georgia elementary school (MMWR, 1985). From May 10 to May 16, an estimated 351 children and staff at the school developed febrile gastroenteritis with 23 children being hospitalized. *Salmonella* Enteritidis was isolated from more than 100 children. Based on a case-control study, illness was associated with consumption of an overnight-refrigerated turkey salad during school lunch.

Eggs are considered to be an important source of *Salmonella*. According to the CSPI eggs on an average contribute to 21 % of foodborne illness outbreaks annually (CSPI, 2001). *Salmonella* Enteritidis is an important pathogen of the layer industry due to its ability to infect hens and ultimately contaminate egg contents (Seo et al., 2000). An outbreak traced to consumption of a nationally distributed ice cream product (Schwan's ice cream) occurred in Minnesota during 1994 (MMWR, 1994). A total of 80 confirmed cases involving *Salmonella* Enteritidis were reported to the Minnesota Department of Health from September 19 to October 10 with 73 % of case-patients reporting consumption of Schwan's ice cream within 5 days of onset of illness. Cases were characterized by diarrhea, abdominal cramps and fever. The company voluntarily stopped distribution and production at its plant in Marshall, MN following investigation. *S.* Enteritidis was isolated from samples of ice cream from households of ill persons.

In June 1995, another outbreak associated with consumption of baked eggs occurred in an Indiana nursing home (MMWR, 1996). Seventy individuals, including the residents and staff, were infected with three residents dying from complications. Thirty-nine cases of *Salmonella* Enteritidis associated with the consumption of baked eggs were confirmed by stool cultures.

During November of 1995, another outbreak was traced to a Thanksgiving dinner at a private home in Nevada (MMWR, 1996). All seven individuals who consumed turkey and stuffing developed abdominal cramps, vomiting and diarrhea. Two people were hospitalized and a third person died. Stool cultures from these three individuals yielded *Salmonella* Enteritidis phage type 13a.

During April and May of 1995, the Idaho Department of Health and Welfare and the Washington Department of Health identified three and nine isolates of *Salmonella* Montevideo, respectively. In April and May of 1996, a total of 11 isolates of *S.* Montevideo were reported in Washington. A case was defined as a culture-confirmed *S.* Montevideo infection in an Idaho or Washington resident with onset of illness during April and May of 1995 or 1996. A total of 23 cases were identified. Handling of chicks was implicated as a health risk, especially for children, as most case-patients were children aged two years or less. An isolate from a 14-month-old child was cultured from blood indicating invasive disease. No common hatchery or sources of feed were identified. But *S.* Montevideo was isolated from fecal samples obtained from two chicks handled by geographically separated patients.

From March to June 1996, the Oregon State Public Health Laboratory also identified 16 cases of *S.* Montevideo (MMWR, 1997). A case-control study involving 11 of the 16 cases revealed previous handling of live poultry (chicks, hens, or roosters). *S.* Montevideo was isolated from two chicks obtained from one of the case-patients.

A similar outbreak of salmonellosis associated with handling live chicks and ducklings was reported in 1999 (MMWR, 2000). In April, the Missouri Department of Health discovered a cluster of *Salmonella* serotype Typhimurium infections with identical PFGE (Pulsed Field Gel Electrophoresis) patterns. A total of 40 cases were identified with symptoms of fever, bloody diarrhea, stomach cramps and vomiting. The Michigan Department of Community Health was notified of an increase in *Salmonella* serotype Infantis infections in May of 1999. From April to July of 1999, a total of 21 cases were reported with these victims also exhibiting diarrhea, fever and vomiting. The young chicks and ducklings associated with the outbreak (88%) were traced to a hatchery. S. Infantis was recovered from environmental samples and from birds of the hatchery, which exhibited the same PFGE pattern as the patient isolates from the outbreak.

In the poultry industry, *Salmonella* infections have a major impact on productivity of poultry through mortality, reduced feed efficiency and reduced weight gain. Salmonella in poultry products alone causes 409,624 cases of foodborne salmonellosis, resulting in an economic loss of $ 6.96 million along with heavy losses to the industry through plant clean-ups and product recalls (Table 2) in cases of outbreaks (Mead et al., 1999; CSPI, 2001).

2.4. *SALMONELLA* IN POULTRY

Salmonellae in poultry are a major source of human infection (Bryan, 1980). This group of bacteria is ubiquitous and has been isolated from various points during poultry production including the breeder flock, newly-hatched chicks, grower birds, feed/feed ingredients, water, processing equipment and the finished raw product (Lahellec et al., 1986; Todd, 1980). Poultry usually acquire salmonellae from the feed and feed ingredients, water, litter and the environment. After the birds are infected, *Salmonella* is conveyed to the processing plant through fecal material on their feet, skin and feathers or through the contents in their gastrointestinal tracts with the carcasses thereby becoming contaminated. Numerous studies have shown that broilers entering processing plants are highly contaminated with salmonellae being firmly attached to poultry skin (Kotula and Pandya, 1995; Lillard, 1989[a]; Lillard, 1989[b]). Broilers are widely recognized reservoirs for *Salmonella* infections in humans due to the ability of the organism to colonize and proliferate in the gastrointestinal tract of the birds and subsequently survive on the carcass during processing (Todd, 1980; Cason et al., 1997).

Table 2: Class I recalls of poultry products contaminated with *Salmonella* in the United States

Product	*Date recalled*	*Origin*	*Distribution*	*Quantity recalled (lbs)*
Chicken nuggets	08 / 03 / 2000	Israel	New York City	6,000
Chicken salad	08 / 20 / 2000	USA	Pennsylvania Delaware	460
Piroshkies	12 / 02 / 1999	USA	San Francisco	22
Chicken nuggets	09 /30 / 1999		New York City	1,800
Cooked poultry-products	12 / 17 / 1998	USA	WV	29,364
Dressing mix	09 / 22 / 1998	USA	Arkansas, Florida, Georgia, Idaho, Texas, Louisiana, Mississippi, Tennessee, Michigan.	500,000

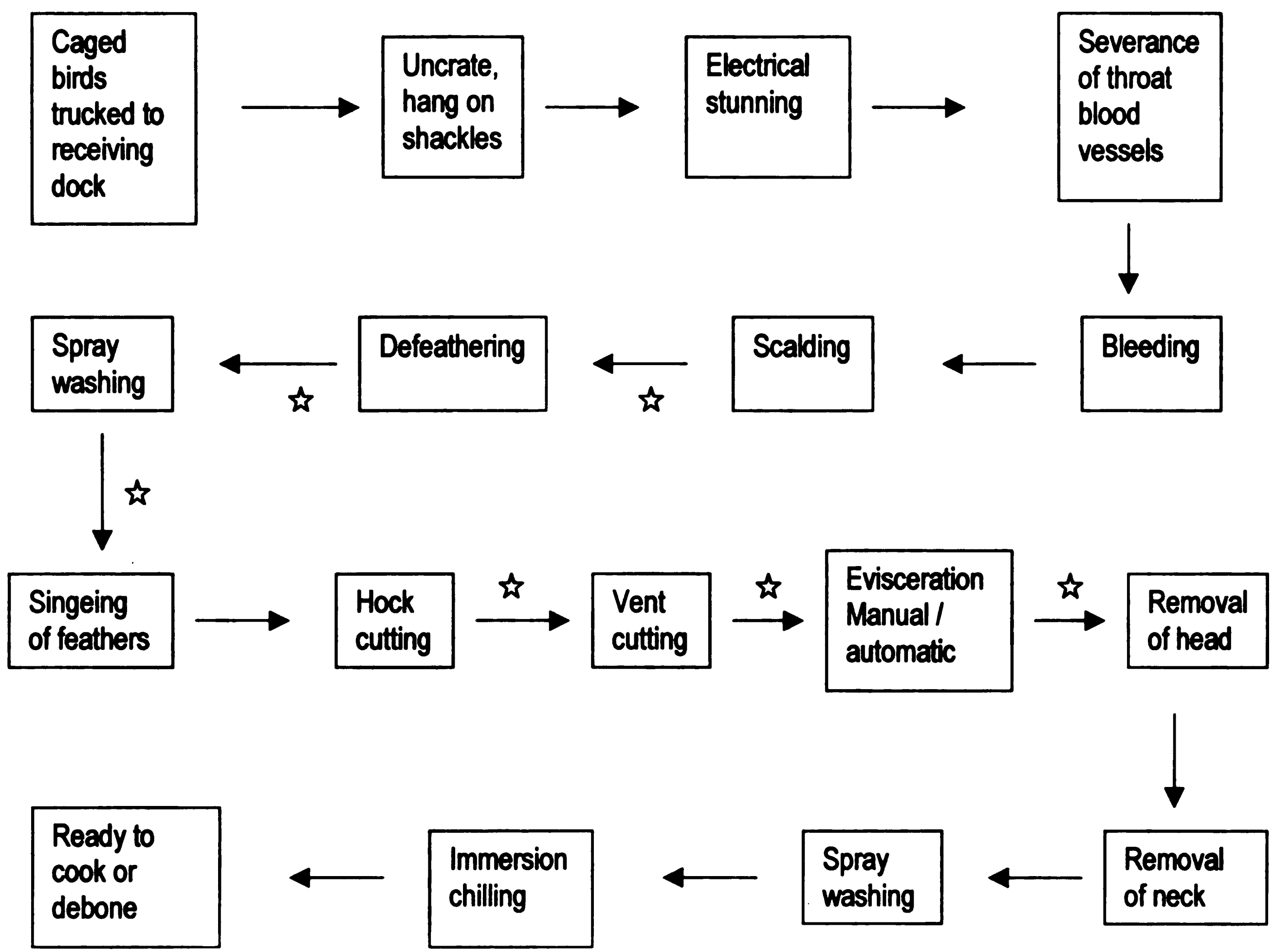
(FSIS-USDA, 1998-2000)

Colonization of chickens with *Salmonella* depends on many factors such as the age of the bird, survival of the organism through the gastric barrier, competing bacteria in the intestinal tract and the effect of antimicrobial feed additives. Milner and Shaffer (1952) were first to report that newly-hatched chicks lack a complex gastrointestinal flora and hence are prone to colonization by enteric bacteria. They also showed that day-old chicks could be infected by ingesting fewer than 5 cells of *Salmonella* with older birds gradually becoming more resistant to infection. Thus, resistance to colonization increases with age as the normal gut flora becomes established.

Hatcheries frequently serve as reservoirs for *Salmonella* with contamination from the hatchery reportedly an important source of *Salmonella* in newly-hatched out chicks, which are most susceptible to intestinal colonization. A single *Salmonella*-contaminated egg can reportedly contaminate other eggs and chicks in the hatching cabinet (Bailey et al., 1994). Another important source of *Salmonella* is the feed. Erwin (1955) reportedly recovered viable salmonellae from commercial poultry feed and since then the role of poultry feed and feed ingredients in dissemination of *Salmonella* has received widespread attention. Jones et al (1991) and MacKenzie and Bains (1976) also isolated *Salmonella* from feed and feed ingredients. Morris et al (1969) showed the presence of *Salmonella* in both feed samples and breeder flocks. During grow-out, the farm environment in which broilers are raised is another primary source of contamination (Blankenship et al., 1993) with the importance of flies in the spread of *Salmonella* also having been documented by Olsen and Hammack (2000) and Bailey et al., (2001).

The contamination rate of broiler carcasses in processing plants and retail outlets also has been studied worldwide with 5% to 100% of such carcasses harboring salmonellae (Todd, 1980; Jerngklinchan et al., 1994; Plummer at al., 1995; Rusul et al., 1996; Carraminana et al., 1997; Uyttendaele et al., 1999; Sackey et al., 2001; Beli et al., 2001; Dominguez et al., 2002). Fecal matter often found on the breasts of caged birds during transportation is considered to be an important source of *Salmonella* in processing plants with broiler carcasses also being cross-contaminated with salmonellae and other bacteria during processing (Lillard, 1990). Cross-contamination of the carcass may occur at many points of contact during poultry processing (Figure 1), particularly during immersion, scalding and chilling. Thus, since the spread of *Salmonella* through integrated broiler operations is complex, multiple control measures need to be implemented at different processing points to control the entry and spread of *Salmonella* into broiler operations.

Figure 1: Main points in a modern poultry processing plant with most probable points at which Salmonella contamination (☆) could occur



2.5. SALMONELLA ENTERICA SEROVAR INFANTIS

Salmonella enterica serovar Infantis, hereafter *S.*Infantis, is one of the more commonly isolated serovars of *Salmonella* from humans and is an important pathogen in the broiler industry. *Salmonella* Infantis belongs to the Paratyphoid group of *Salmonella*, which includes salmonellae that frequently infect or colonize the gut of many warm- and cold-blooded animals including humans. Wheeler and Borman (1943) were first to isolate *S.*Infantis from a four-month-old girl in the United States. Levy et al., (1975) described an outbreak of salmonellosis in Middleton, MN in which *S.*Infantis was one of three serovars identified. In this outbreak 125 of 173 (72.25%) individuals who attended a picnic and/or smorgasbord prepared by a bar/restaurant developed symptoms of diarrhea, abdominal cramps, chills, fever, nausea, vomiting and headache. Potato salad and chicken dressing were implicated as vehicles for the outbreak. The economic impact of this 1973 outbreak estimated as $ 28,733.

*S.*Infantis was the cause of another outbreak in Finland involving railway and airline passengers in 1986 (Hattaka, 1992). This outbreak was traced to an employee who was an asymptomatic carrier of *S.*Infantis who had contaminated breakfast / egg sandwiches during preparation.

Kohl and Farley (2000) described a 1998 outbreak of salmonellosis that occurred among 63 wedding participants. A commercially cooked rice-dressing mix product contaminated with *S.*Infantis was identified as the source of infection. The rice-dressing mix contained pork, pork liver and chicken gizzards in combination with spices and other flavorings. Investigations showed that the product had likely become contaminated at the plant through contact with a pump in the production line. Consequently, on September 22, 1998, the USDA issued a Class I recall for all products produced before September 15, 1998.

*S.*Infantis is frequently isolated from chicken carcasses and derived products (Lammerding et al., 1988) and has also been recovered from insects in poultry houses (Jones et al., 1991). According to McBride et al., (1980), *S.*Infantis was the predominant serovar isolated from one poultry processing plant. They observed that *Salmonella* contamination was most common before scalding compared to post-evisceration and post-chilling sites. More recently, Uyttendaele et al., (1998) reported an isolation rate of 6.6% for *S.*Infantis in chicken carcasses and derived products during a four-year study of *Salmonella* in poultry carcasses and their products sold in Belgium. Bailey et al. (2001) have also recovered *S.*Infantis from different points in the production chain including the carcass rinse, transport coop, paper pads used in transportation trays, litter, fly strips, and caecal droppings.

2.6. CONTROL OF SALMONELLA IN POULTRY

Salmonellae are ubiquitous bacteria with broilers representing a highly important reservoir of salmonellae in the human diet. The problem of human salmonellosis cannot be overcome until *Salmonella* infections in birds are under control. In an integrated poultry operation, control of *Salmonella* is often very complicated as there are many potential sources of contamination such as the birds, feed, water, rodents, flies, insects, farm, transportation, and the processing plant environment. Much research has been conducted to eliminate salmonellae from the various steps in poultry production and processing.

Salmonellae colonize the intestinal tract of chickens with the crop and the caecum being the main sites of colonization (Hinton et al., 1990; Soerjadi et al., 1981). Minimizing cross-contamination of the dressed carcass with intestinal contents of birds during processing helps improve the microbiological quality of poultry meat (Bailey, 1993). Therefore, reduction of *Salmonella* in broilers during the grow-out period is a very important issue. Commonly used control measures in broiler production include addition of antimicrobials at sub-therapeutic levels to poultry feed and the use of competitive exclusion products. However, other control measures such as vaccination, feeding of probiotics, feeding of mannanoligosaccharides, and addition of buffered propionic acid also have been investigated (Izat et al., 1990; Methner et al., 1999; Spring et al., 2000; Tellez et al., 2001).

2.6.1. PRE-HARVEST CONTROL MEASURES

Salmonella control is very difficult in integrated poultry operations as there are many sources of contamination such as feed, water, hatchery, farm environment and processing environment. The gastrointestinal tract of poultry is one of the primary sources of contamination during processing (Bailey, 1993). To reduce the contamination of *Salmonella* on processed carcasses, Salmonella-free birds must be delivered to the processing plants. Salmonella in the intestinal tract of birds can be reduced or eliminated by pre-harvest control measures, some of which are described below.

2.6.1.1. POULTRY FEED ADDITIVES

The poultry industry employs different preventative measures to control economically important diseases such as coccidiosis and different bacterial infections that negatively impact the performance birds. Addition of antimicrobials and anticoccidiostats is frequently used as a preventative measure. As their full potential for disease control was discovered, the demand for antibiotic feed supplements increased. Supplementation of feed with antibiotics may occur for any of the following purposes (1) to improve the growth rate / weight gain of animals (2) disease prophylaxis and (3) disease treatment. Antibiotics mixed at low levels in poultry, cattle, swine and sheep rations reportedly cause increased weight gain and a decrease in shedding rates of certain pathogenic organisms including *Clostridium* and *Salmonella* (Engberg et al., 2000; Bolder et al., 1999).

In broiler production, asymptomatic *Salmonella* infections are an important problem, which cannot be solved completely by strict hygienic practices or by the use of antimicrobials in feed. Antimicrobials added to feed have been used both in the treatment and prevention of *Salmonella* infections in chickens. In one study, addition of chlortetracycline at sub-therapeutic levels to feed reduced mortality in chicks infected with *S.*Typhimurium (Quarles et al., 1977). However, while Nivas et al. (1976) showed that chlortetracycline decreased fecal shedding of *S.*Typhimurium in turkey poults, higher isolation rates were obtained from the caecal junction and liver. In other work, Williams (1985) showed a lower incidence of salmonellosis in chickens fed a combination of neomycin and oxytetracycline. However, antimicrobials that were effective against *S.*Infantis *in vitro* were ineffective in treating *in vivo* intestinal infections (Seuna and Nurmi., 1979). Virginiamycin, another antibiotic effective against primarily gram-positive organisms, had no effect on shedding and antibiotic resistance patterns of *S.*Typhimurium when added to poultry feed (Abou-Youssef et al., 1982).

Over the last five years, the practice of supplementing feed with sub-therapeutic levels of antibiotics has become highly controversial, largely due to the rapid emergence in multi-antibiotic resistant serovars of *Salmonella* and other foodborne pathogens. Antimicrobials tend to destroy the normal bacterial flora of the intestine and can thereby lead to enhanced colonization by opportunistic pathogens (Rantala, 1974). Seuna et al., (1980) reported the reappearance of salmonellae in poultry when treatment with antibiotics such as neomycin, oxytetracycline, polymyxin B, trimethoprim, and sulfadiazine alone or in various combinations was stopped. They also observed more infected chickens when the antibiotic treatment was stopped. While ionophore antibiotics are widely used as poultry feed additives to control coccidiosis, they are also considered to have growth promoting properties (Elwinger et al., 1998). According to Engberg et al. (2000), supplementation of feed with ionophore antibiotics such as zinc bacitracin and salinomycin, either alone or in combination, significantly reduced natural populations of *Clostridium perfringens* and *Lactobacillus salivarius* in the intestines of broilers. It was suggested that the mode of action for these feed additives is mainly related to either the inhibition of certain toxin-producing intestinal bacteria or those bacteria that compete with the host for easily available nutrients (Coates et al., 1963). Monensin, one of the most commonly used ionophore antibiotics in poultry production, is mainly used as a coccidiostat. Hence, not much research has been conducted on the effects of monensin on *Salmonella* infections in poultry. Studies done by Holmberg et al (1984) showed that chickens experimentally infected with *S.*Infantis had higher caecal counts when fed diets containing avoparcin and monensin. Although sub-therapeutic use of broad spectrum antibiotics can decrease shedding of *Salmonella*, the effects of coccidiostatic antibiotics such as monensin have yet to be studied.

2.6.1.2. ANTIBIOTIC RESISTANCE AND SALMONELLA

The widespread occurrence of *Salmonella* species in the natural environment coupled with intense husbandry practices applied to the broiler industry have increased the prevalence of salmonellae in the food chain to the point where *Salmonella* is one of the leading causes of bacterial foodborne illness (D'Aoust 1989). The administration of sub-therapeutic doses of antibiotics as feed additives to domestic livestock is practiced worldwide. Such doses of antibiotics are lower than those required for treating infections in farm animals but are sufficiently high to suppress the growth of normal intestinal microflora. This agricultural practice leads to the development of selective pressures which in turn can lead to the emergence and spread of resistant salmonellae in products for human consumption (Holmberg, 1987; Dupont and Steele, 1987; Threlfall et al., 2000; Usera et al., 2002). Poultry is often found to be a major reservoir of resistant salmonellae (Rajashekara et al., 2000). Foods harboring resistant strains present an increased level of risk to humans as they can cause systemic infections that often result in serious complications.

Emergence of multi-antibiotic resistant bacteria has become an increasing problem worldwide with infections from such organisms becoming increasingly difficult to treat. The widespread prophylactic use of antibiotics in human and veterinary medicine, combined with sub-therapeutic use in animal feed has led to the emergence of antibiotic resistant strains of *Salmonella*. Many strains isolated from poultry, especially those fed with antibiotics, exhibit multiple resistances to antibiotics such as ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline. These strains can then progress through the food chain to humans via poultry and poultry products (Arvanitidou et al., 1998).

In 1981, approximately 2% of the normal "wild type" bacterial population was resistant to any given antibiotic (Novick et al., 1981). However, when bacterial populations from animals are regularly exposed to antibiotics, up to 10% of the population can acquire resistance. These resistant bacteria are often shed in feces, where they share extrachromosomal antibiotic resistance plasmids (r-plasmids) or transposons with native bacteria and may be spread to other animals. In addition, antibiotics that accumulate in animal tissues can lead to further resistance in humans when the product is ingested (Siegel at al., 1974). Genes coding for resistance to several antibiotics can be located within the same transposon or plasmid. Aarestrup et al. (2001) showed that the use of a single antibiotic as a treatment also could select for resistance to other antibiotics when the genes are located in the same mobile genetic element ("linked resistance").

The emergence of antimicrobial-resistant salmonellae is a problem not solely confined to the United States, where it has been increasing. In 1979, 16% of the *Salmonella* isolates were found to be resistant to at least one antimicrobial agent, whereas in 1989 and 1990, 29% and 37% of all *Salmonella* isolates were resistant to at least one antimicrobial agent (Riley et al., 1984; MacDonald et al., 1987; Lee et al., 1994). The United States experienced its first outbreak of multidrug-resistant *Salmonella enterica* serovar Typhimurium definitive phage type 104 (Typhimurium DT 104) in 1996 (MMWR April 11, 1997). This outbreak occurred in Nebraska during October 1996 among school children. Investigations identified the cause as chocolate milk poured from cartons during lunch at school. Stool cultures from affected children yielded *S.*Typhimurium R-type ACSSuT and phage typing confirmed all isolates as DT 104.

Threlfall et al. (1994) reported that the DT 104 isolates from the United Kingdom contained chromosomally integrated genes conferring resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT). Multidrug resistance is associated with the presence of two integrons found in the chromosomal DNA of DT 104 R-type ACSSuT isolates (Threlfall et al., 1994; Sandvang et al., 1998; Briggs et al., 1999). Additional resistance to trimethoprim and ciprofloxacin also has been reported in some DT 104 strains (Threlfall et al., 1997). Though primarily a cattle pathogen, multidrug resistant strains of DT 104 have been recovered from other forms of domestic livestock including swine and poultry (Besser et al., 1997; Rajashekara et al., 2000). Infected animals and consumption of contaminated chicken, pork sausage and meat paste have been identified as potential sources of DT 104 infection in England and Wales (Wall et al., 1994).

2.6.1.3. COMPETITIVE EXCLUSION

Competitive exclusion (CE), a widely used method for reducing fecal shedding of *Salmonella* in poultry, is a technique whereby newly hatched chicks (day-old) are exposed to the gastrointestinal flora of adult birds. Colonization of the gastrointestinal tract with normal gut flora from healthy birds discourages colonization by salmonellae. The benefits derived from competitive exclusion may be due to either the production of short-chain volatile fatty acids in the caecum (Bailey et al., 1988; Corrier et al., 1990; Nisbet et al., 1996) or competition for *Salmonella* attachment sites on the intestinal mucosa (Soerjadi et al., 1981[a]; Hinton et al., 1990; Nisbet et al., 1993). Several researchers including Blanchfield et al., (1982) and Stavric et al., (1985) further assessed the effectiveness and practical applications of competitive exclusion treatments. Blankenship et al., (1993) and Bailey et al., (2000) found that a two-step treatment of broiler chicks with a mucosal competitive exclusion culture (MCE) containing intestinal mucosal scrapings of pathogen-free adult broiler chicks was effective in reducing *Salmonella* colonization. Treatment consisted of spraying the chicks in the hatchery with the MCE culture after which the culture was ingested in the drinking water. Competitive exclusion products alone or in combination with fructooligosaccharide (Fukata et al., 1999) or vaccination (Methner et al., 1999) to reduce bacterial colonization in poultry particularly of salmonellae were also studied. Feeding low doses of fructooligosaccharide in combination with CE treatment decreased *Salmonella* colonization in chicks. Although the competitive exclusion treatment effectively reduced *Salmonella*, other challenge studies have discounted the use of commercially available CE cultures to protect against infection by *Salmonella* (Stavric et al., 1992).

2.6.1.4. SODIUM CHLORATE

The need to develop more potent antimicrobial agents against *Salmonella* has increased in recent years due to the increasing number of foodborne outbreaks and the continuing emergence of multi-antibiotic resistant strains. One such alternative approach that is gaining much attention is the feeding of sodium chlorate to domestic livestock to reduce intestinal colonization by *Salmonella* and *Escherichia coli* O157:H7.

In the gastrointestinal tract of animals, nitrate can be reduced to nitrite anaerobically by some bacteria that possess the intracellular enzyme nitrate reductase (Alaboudi, 1982). As is true for most members of the Family *Enterobacteriaceae*, salmonellae possess respiratory nitrate reductase activity. This enzyme can catalyze the intracellular reduction of chlorate to cytotoxic chlorite which is lethal to those bacteria possessing respiratory nitrate reductase activity (Stewart, 1988). In contrast, much of the normal gastrointestinal flora does not possess respiratory nitrate reductase and is therefore not affected. *In vivo* studies have shown that sodium chlorate is bactericidal to *E.coli* O157: H7 and *Salmonella* Typhimurium DT 104 with the beneficial gut microflora remaining unaffected (Anderson et al., 2000). Caecal populations of *Salmonella* were significantly reduced in weaned pigs treated orally with a sodium chlorate solution (Anderson et al., 2001). Similarly oral administration of sodium chlorate was found to reduce gut concentrations of *E. coli* O157:H7 in experimentally infected pigs and wild-type *E. coli* concentrations in uninoculated pigs (Anderson et al., 2001).

2.6.1.5. OTHER PRE-HARVEST CONTROL MEASURES

Feeding of complex sugars such as mannose or lactose to birds also can inhibit intestinal colonization by salmonellae. Supplementation of feed with mannanoligosaccharide reportedly decreased *S.*Typhimurium populations about 25-fold in chicks (Spring et al., 2000). Consumption of lactose-supplemented feed throughout the growing period of broilers also significantly reduced the number of salmonellae in the caeca (Corrier et al., 1990).

Another control measure gaining much attention is the feeding of probiotics. Avian *Lactobacillus* species were shown to prevent *S.*Typhimurium from adhering to the crop mucosa and also reduce caecal colonization (Fuller, 1977; Soerjadi et al., 1981[b]). Studies by Tellez et al., (2001) demonstrated that the treatment of chicks with the avian-specific probiotic Avian Pac Plus® significantly decreased caecal colonization and organ invasion by *S.*Enteritidis.

Feeding of a buffered propionic acid product (53.5% propionic acid, 9.5% ammonium hydroxide, 11.5% 1, 2-propanediol, and 25.5% water) in drinking water to broiler chickens also significantly reduced the number of salmonellae on post chill carcasses (Izat et al., 1989; Izat et al., 1990). Another study by Byrd et al., (2001) showed that the use of 0.5% lactic acid in drinking water during pre-transport feed withdrawal time (8hrs) caused a significant reduction in the incidence of *Salmonella* in the crop and thereby a reduction in the pre-chill carcass rinse.

2.6.2. POST HARVEST CONTROL MEASURES

Decontaminating the final raw product is one of the most effective approaches in reducing poultry-borne salmonellosis. The ideal method of decontamination should not change appearance, smell, taste or nutritional properties; should not leave residues and should be environment-friendly as well as cheap and easy to use (Corry et al., 1995). Some of the decontamination techniques used in poultry processing plants are described below.

2.6.2.1. ACIDS:

The undissociated forms of lactic, acetic, and other acids are widely recognized for their antimicrobial activity (Mountney and O'Halley 1965; Stern et al., 1985; Colberg and Izat 1988). Lactic acid was shown to extend the shelf-life of processed broilers; however, concentrations of organic acids that effectively decontaminated poultry carcasses generally caused unfavorable sensory changes (Izat et al., 1989). Addition of 1% acetic acid to poultry scald water caused instantaneous bacterial death and reduced cross contamination (Okrend et al., 1986). The effect of acids in combination with other compounds was also studied. Washing chicken wings with a solution containing 0.5% lactic acid and 0.05% sodium benzoate was found to effectively reduce the number of *Salmonella* and thus enhance product safety and shelf-life (Hwang and Beuchat, 1995).

2.6.2.2. TRISODIUM PHOSPHATE:

Trisodium phosphate is an antimicrobial compound approved for use in poultry processing facilities by the USDA-FSIS. When used at a concentration of 8%, this compound had a pH>12 and was active against gram-negative bacteria on the skin of chickens (Corry et al., 1995). One such product marketed as "Avgard®" is used as a dip immediately after water-chilling or before air-chilling (Corry et al., 1995). Whyte et al. (2001) also reported significant microbial reductions on broiler carcasses using trisodium phosphate.

2.6.2.3. CHLORINE

Another antimicrobial agent commonly used in poultry processing is chlorine. Used for chilling carcass, chlorine is converted to its active form hypochlorous acid (pH 6.5 – 8.0) in water, which destroys pathogenic organisms on poultry carcasses. Addition of chlorine to poultry chill water was shown to reduce cross-contamination by *Salmonella* in chill tanks (James et al., 1992; Morrison et al., 1985).

2.6.2.4. CHLORINE DIOXIDE

Another form of chlorine known as chlorine dioxide that has been approved by the FDA in 1995 (USDA, 1995) is being used by many poultry processors as a disinfectant in chiller water. Chlorine dioxide, a gas produced as a result of the reaction between hydrochloric acid and sodium chlorite can be incorporated in de-ionized water. Chlorine dioxide acts on the cell membrane of bacteria resulting in a loss of membrane permeability and thus producing non-specific oxidative damage to the cell membrane (Berg et al., 1986). Chlorine dioxide was found to reduce microbial populations in poultry chiller water (Tsai et al., 1992; Lillard, 1979). It has also been shown to destroy a wide variety of microorganisms including spores resistant to chlorine treatment (Richardson et al., 1994). Other advantages of this compound include its effectiveness over a wide range of pH, and in the presence of high levels of organic matter and slow dissociation in water compared to chlorine (White, 1972).

2.6.2.5. OZONE:

Ozone is prepared by passing gaseous oxygen or dry air through a high voltage electrical field, which is then dissolved in water. The ozonated water attacks the bacterial membrane and certain amino acids, disrupting the normal cellular activity and killing the microorganism. Ozone has been tested for disinfecting poultry chiller water, poultry carcasses (Sheldon and Brown, 1986), and eggs, as well as the hatchery and hatching eggs (Whistler and Sheldon, 1989). When carcasses were chilled in water containing ozone at 3.0 to 4.5 ppm for 45 minutes, Sheldon and Brown (1986) reported that microbial counts decreased by more than 2 logs. Yang and Chen (1979) also reported similar results using ozone at a concentration of 37.7 mg / liter.

2.6.2.6. ULTRAVIOLET RADIATION:

In the food industry, UV light is commonly used to disinfect packaging surfaces. Sumner et al. (1995) reported that UV radiation (2000 μW sec cm^{-2}) effectively destroyed *S.*Typhimurium on agar plates and poultry skin; but the bacterial reduction on poultry skin was less effective. Yndestad et al. (1972) also found that UV radiation (10,000 μW sec cm^{-2}) decreased the superficial microflora on poultry carcasses but did not have any prolonged effect on shelf-life.

2.6.2.7. ELECTRICITY:

Effects of electrical stimulation in reducing the number of *S.*Typhimurium attached to chicken legs were studied by Slavik et al. (1991). Their results indicated that electrical stimulation killed bacteria in solution and reduced the number of salmonellae attached to chicken legs when the legs were attached to anodes. However, slight damage to the meat was reported when the chicken legs were connected to the electrodes.

2.6.2.8. IRRADIATION:

This method has been approved by more than 40 countries and also by the FDA in the United States to reduce the incidence of harmful pathogens on meat and poultry products. The three types of irradiation currently permitted to be used on raw meat and poultry include gamma irradiation, electron beams, and X-ray (CFR, Title 21, vol 3, part 179). Gamma irradiation at a dose of 3.0 KGy inactivated 99.9% of common foodborne pathogens (Frenzen et al., 2001). However in the United States, irradiation of poultry meat was accepted by only 49.8% of the population. If not applied carefully at the correct temperature and dose, irradiation can lead to undesirable textures, off-flavors and inadequate inactivation of pathogens (Mucklow and Cross, 2002). This method is currently being test marketed with ground beef products.

2.6.2.9. OTHER POST-HARVEST CONTROL MEASURES:

The antibacterial effect of the lactoperoxidase (LP) system on growth and survival of *S.*Typhimurium on poultry was studied by Wolfson et al., (1994). They found that an LP system treatment consisting of lactoperoxidase (1μg/ml); potassium thiocyanate (5.9 mM) and hydrogen peroxide (2.5 mM) in water reduced levels of *Salmonella* on inoculated chicken legs. They reported a reduction of 80.6% when treated for 15 min at 60°C and 13.6% when treated for 30 min at 25°C.

EFFECTS OF MONENSIN AND SODIUM CHLORATE ON *SALMONELLA* INFANTIS COLONIZATION IN BROILERS

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CHAPTER 3

EFFECTS OF MONENSIN AND SODIUM CHLORATE ON *SALMONELLA* INFANTIS COLONIZATION IN BROILERS

3.1. ABSTRACT:

Intestinal colonization and fecal shedding of *Salmonella* in broilers has been an on-going food safety concern. Any reduction of *Salmonella* colonization in the gastrointestinal tract of broilers will ultimately lead to a decrease in carcass contamination during processing and thus yield a safer product. Two studies were conducted to determine the effects of monensin and sodium chlorate on *Salmonella* Infantis colonization in broilers. In the first study, 210 *Salmonella*-free birds were fed either an antimicrobial-free control diet or a treatment diet containing monensin. All chicks were orally infected on day 3 with 1 X 10^8 CFU of *S.* Infantis. When caecal colonization by *S.* Infantis was evaluated on days 7, 10, 14, 21, 28, 35, and 42, monensin failed to reduce *S.* Infantis colonization. In the second study, 150 *Salmonella*-free broilers were divided into 4 groups (3 treatment groups and a control group) and similarly infected on day 3. The three treatment groups received a commercial sodium chlorate solution *ad libitum*, on day 10, 20, or 38 for four consecutive days followed by drinking water whereas the control group received only drinking water. Caecal colonization and liver invasion by *S.* Infantis in all groups were assessed on days 7, 10, 14, 17, 20, 24, 27, 30, 38, and 42 with fecal swab samples also analyzed on days 11, 12, 13, 15, 16, 18, 19, 21, 22, 23, 25, 26, 28, 29, 39, 40, and 41. Birds receiving sodium

chlorate on days 10 and 20 had significantly lower caecal populations of *S.* Infantis four days after treatment ($P<0.02$ and $P<0.0001$, respectively) compared to the untreated control group with these lower populations persisting up to seven days after treatment. Fecal shedding of *S.* Infantis also decreased >1 and >2 logs three days after sodium chlorate treatment was begun on days 10 and 20, respectively, with no effect observed in birds treated on day 38. While no difference in liver invasion by *S.* Infantis was seen, lower caecal populations as well as reduced fecal shedding of *S.* Infantis indicates that sodium chlorate may be potentially useful for decreasing the incidence of *Salmonella* in broilers entering poultry processing facilities.

3.2. INTRODUCTION:

Each year the United States experiences an estimated 1.4 million cases of foodborne salmonellosis, making *Salmonella* a leading cause of the 76 million cases of foodborne illnesses reported annually (Mead et al., 1999). According to the Center for Science in the Public Interest (CSPI, 2001), about 29% of these outbreaks are caused by poultry products, which makes poultry the most important vehicle for foodborne salmonellosis.

Salmonella Infantis is among the 10 most commonly reported strains of *Salmonella* isolated in the United States (CDC, 2000). In 1999 the State of Michigan experienced an increase in S. Infantis infections with this serovar recovered from both patients and poultry farm environments (Wilkins et al., 2002) with subsequent investigations linking this outbreak to the handling of chicks and ducklings.

Broilers are widely recognized reservoirs for *Salmonella* infections in humans. The primary source of *Salmonella* in the poultry processing environment is the gastrointestinal tract of broilers where the organism proliferates (Hinton et al., 1990) with cross-contamination of the carcass after evisceration leading to further contamination of the processing environment. Efforts that minimize cross-contamination of poultry carcasses with intestinal contents will improve the microbiological quality of carcasses (Bailey, 1993). Therefore, control measures aimed at reducing *Salmonella* in the gastrointestinal tract will likely lead to a reduction in poultry-associated salmonellosis.

Monensin, an ionophore antibiotic, is commonly used as a feed additive in the poultry industry for the control of coccidiosis. However, the impact of monensin use alone on shedding *Salmonella* has not been reported. A more recent approach for controlling *Salmonella* in domestic livestock is feeding of sodium chlorate which has been shown to minimize *Salmonella* colonization in pigs (Anderson et al., 2001). Hence, the purpose of this study was to investigate the effects of monensin and sodium chlorate on *S.* Infantis colonization and shedding in broilers.

3.3. MATERIALS AND METHODS

3.3.1. EFFECT OF MONENSIN ON *S.* INFANTIS IN BROILERS

A total of 70 newly-hatched *Salmonella*-free broiler chicks were randomly allotted to one of two groups of 35 chicks. One group received feed containing monensin (treatment group) whereas the other group received antimicrobial-free feed. Caecal samples were collected from five randomly selected birds on days 7, 10, 14, 21, 28, 35, and 42 and examined for numbers of *S.*Infantis with the experiment replicated three times.

3.3.2. CONTROL OF *S.* INFANTIS USING SODIUM CHLORATE

Fifty *Salmonella*-free broiler chicks were randomly allotted to one of three treatment groups (12 birds each) or a control group (20 birds). Treatment groups received sodium chlorate solution *ad libitum* for four days in place of drinking water while the control group received only drinking water. The three treatment groups were given sodium chlorate solution beginning on day 10, 20 and 38. Two birds from each group were sacrificed at random after which caecal and liver samples were quantitatively examined for *S.*Infantis on days 7, 10, 14, 17, 20, 24, 27, 30, 38, and 42. In addition, cloacal swab samples were randomly taken from two birds in each group on days 11, 12, 13, 15, 16, 18, 19, 21, 22, 23, 25, 26, 28, 29, 39, 40 and 41, and examined for numbers of *S.*Infantis. This experiment was also replicated three times.

3.3.3. *SALMONELLA* INFANTIS CULTURE

Salmonella Infantis strain 99 EN 151, originally isolated in 1999 from an infected poultry flock in Michigan, was obtained from the Michigan Department of Community Health (MDCH, Lansing, MI). The isolate was maintained on a trypticase soy agar slant at 4°C and transferred four times in trypticase soy broth (35°C/24h) (Becton Dickinson, Cockeysville, MD) before use. After the last incubation, populations were determined by serial dilution in buffered peptone water 0.1% (w/v) followed by spiral plating (Autoplate® 4000; Spiral Biotech Inc; Bethesda; MD) on Xylose Lysine Desoxycholate (XLD) agar (Difco Laboratories, Detroit, MI). On the day of oral challenge, the overnight culture was centrifuged (Sorvall®, Newtown, CT) at 10,000 x g / 10 minutes / 4°C and resuspended in buffered peptone water (Difco) to a final concentration of 1×10^8 CFU/0.25ml with the viable cell count confirmed by spiral plating on XLD agar.

3.3.4. CHICK HATCHING AND HOUSING

Salmonella-free broiler eggs were obtained from Aviagen (Huntsville, AL) and refrigerated at 4°C. The following day, eggs were set for incubation at the Michigan State University (MSU) Poultry Research & Teaching Center in an incubator (Petersime model # 5) at 37.5°C dry bulb and 28.9°C wet bulb for 17 days. On day 18, all eggs were candled and the fertile eggs were transferred to a hatching cabinet (Petersime model) at 37.5°C dry bulb and 32.2°C wet bulb. The newly hatched chicks were randomly assigned to one of two or one of four groups to receive monensin or sodium chlorate, respectively, and housed in brooder cages in the University Lab Animal Resources containment facility. Before infecting the chicks with *S.* Infantis on day 3, caecal samples from nine broiler chicks per group were analyzed to confirm that the chicks were free of *Salmonella*. After three weeks, the birds were transferred to cages for the remainder of the study. Room temperature ranged from 18.3 to 23.9°C. All animal related aspects of these experiments were approved by the Michigan State University All University Committee on Animal Use and Care.

3.3.5. TREATMENT WITH MONENSIN AND SAMPLING

Day-old *Salmonella*-free broiler chicks were randomly allotted to one of two groups, each containing 35 birds. The treatment group was fed a diet containing monensin (Coban-20®; Elanco, Indiana) which was added at the rate of 100 g/ton of feed (100 mg/Kg) whereas the control group received feed without monensin. The diet, which met National Research Council requirements for poultry (NRC, 1994), was prepared at the MSU feed mill. On day 3, all chicks were infected with *S.*Infantis (10^8 CFU/bird) by administering 0.25ml of the buffered peptone suspension by oral gavage. All birds were given feed and water *ad libitum* throughout the experiment. On day 7, five chicks per replicate (15 chicks from each group) were randomly selected from each group and humanely euthanized. The caeca were aseptically removed, placed in individual Whirl-Pak® bags (Nasco, Fort Atkinson, WI) and transported to the laboratory on ice within 1 h of collection. The same procedure was repeated on days 10, 14, 21, 28, 35, and 42, with all samples analyzed for numbers of *S.*Infantis.

3.3.6. TREATMENT WITH SODIUM CHLORATE AND SAMPLING

Fifty day-old *Salmonella*-free broiler chicks were randomly allotted to one of four groups consisting of three treatment groups (day 10 treatment group, day 20 treatment group and day 38 treatment group) and a control group. On day 3, all chicks were infected with *S.*Infantis (10^8 CFU/bird) by administering 0.25ml of the buffered peptone suspension by oral gavage. The control group received only drinking water, whereas birds in the treatment groups were given a commercial sodium chlorate solution containing 0.16% (w/v) sodium chlorate; 0.18% (w/v) sodium lactate and 0.021% (w/v) sodium nitrate (EKA Chemicals; Marletta; GA) *ad libitum*, on days 10, 20, and 38 in place of drinking water. The treated birds were maintained on the sodium chlorate solution for four consecutive days and then switched back to drinking water. All birds were given an antimicrobial-free feed *ad libitum* for the duration of study. Three days before treatment on day 10, caecal and liver samples were aseptically collected from two birds in each replicate (six birds from each group), transported on ice to the laboratory within 1 h of collection and examined for *S.*Infantis. Similar samples were also similarly collected after treatment on days 14, 17, 20, 24, 27, 30, 38 and 42 and examined for *S.*Infantis. On days 11, 12, 13, 15, 16, 18, 19, 21, 22, 23, 25, 26, 28, 29, 40, and 41, cloacal swab samples were collected at random from birds to determine the fecal shedding pattern of *S.*Infantis with the sodium chlorate treatment. On day 42, the body weights of birds from all the four groups were recorded before sacrifice.

3.3.7. MICROBIOLOGICAL ANALYSIS

Caecal and liver samples collected in individual Whirl-Pak® bags were weighed, diluted 1:10 in lactose broth (Difco), minced with a pair of flame-sterilized scissors and homogenized in a Stomacher (Seward Stomacher® 400;

London, England) for 2 minutes. A 1-ml aliquot from each bag was serially diluted in 0.1% peptone water and spiral-plated on duplicate plates of Hektoen Enteric (HE) agar (Difco) and XLD agar (Difco) with the lactose broth enrichment incubated at 35°C for 24 ± 2 h. After 24 ± 2 h of incubation at 35°C, colonies resembling *Salmonella* (black, round and glistening on XLD and HE) were counted as *S*. Infantis. In cases where salmonellae were not observed by direct plating, 1ml aliquots of the 24 h-old lactose broth enrichment were transferred to 10 ml of selenite cysteine broth (Difco) and 10 ml of tetrathionate broth (Difco) and incubated for 24 ± 2 h at 35°C. After incubation, the contents of each tube were streaked to duplicate plates of HE and XLD agar. These plates were again examined for the presence of typical colonies of *Salmonella* following 24 ± 2 h of incubation at 35°C. All cloacal swab samples, which contained 0.2 g of fecal material, were diluted in 10 ml of lactose broth and analyzed as described for caecal and liver samples. Selected isolates were streaked to trypticase soy agar containing 0.6 % yeast extract (Difco) for purification and then confirmed as *S*. Infantis by serotyping at the MDCH.

3.3.8. STATISTICAL ANALYSIS

A repeated measures two-way Analysis of Variance was performed on the data using the Statistical Analysis System (Proc ANOVA, SAS© Version 8, SAS Institute, Inc., Cary, NC). A type III F test was used to assess overall effects of treatments. The least square means were compared using Fisher's LSD (Least Significant Differences) and the significance of mean differences was judged using the t-test. Since the lowest detectable limit by direct plating for cloacal swab samples was 2.7 log CFU/g, all plates which were positive after enrichment were assigned a maximum possible value of 2.7 log CFU/g for statistical purposes.

3.4. RESULTS

3.4.1. TREATMENT WITH MONENSIN

Following oral infection, caecal samples collected from both groups of birds on day 7 contained 7.0 log CFU/g with these counts decreasing from day 7 to day 42 (Table 3.1). In both groups, counts on day 10 were lower ($p < 0.5$) than those on day 7 with 6.5 log CFU/g for the control group and 6.4 log CFU/g for monensin-treated group (Figure 3.1). This decrease in the caecal count was only temporary with *S.* Infantis populations remaining relatively constant throughout the remainder of the study. For the control group, caecal *S.* Infantis counts on day 42 were 6.2 log CFU/g which were only about 0.3 log lower than those observed at day 10. The monesin-treated group yielded a caecal count of 5.8 log CFU/g on day 42 which was 0.6 log lower compared to day 10. Overall, no significant difference in the numbers of *S.* Infantis retrieved from the caeca of monensin-treated birds was observed when compared to the untreated controls.

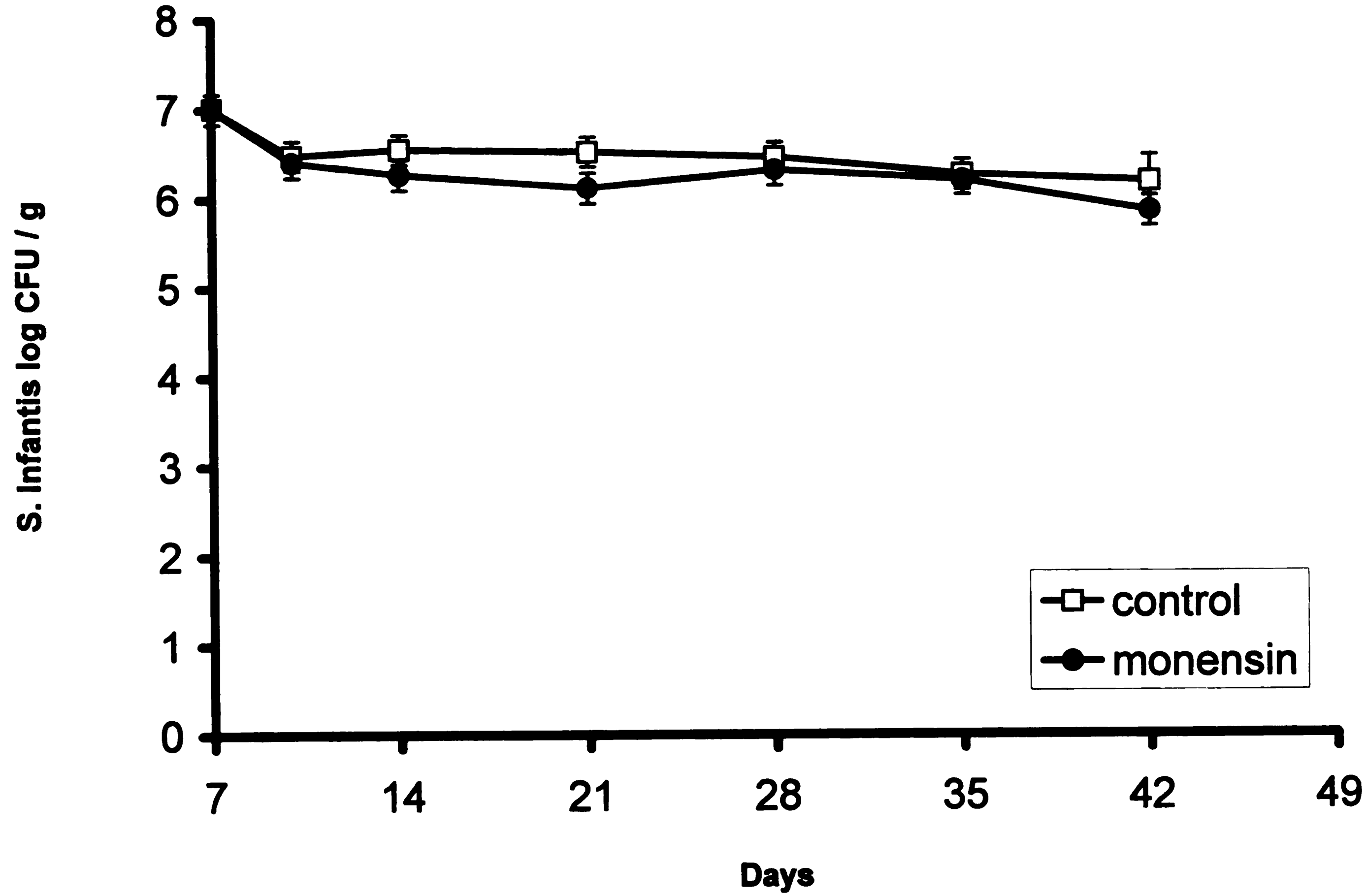
Table 3.1 Mean Caecal populations of *S.* Infantis (CFU/g) in birds raised on a diet with and without monensin

Age of birds	Monensin[1]	Without monensin[1]
7	7.0±0.17[a2]	7.0±0.17[a]
10	6.4±0.17[b]	6.5±0.17[b]
14	6.3±0.17[b]	6.5±0.17[b]
21	6.1±0.17[b]	6.5±0.17[b]
28	6.3±0.17[b]	6.5±0.17[b]
35	6.2±0.17[b]	6.3±0.17[b]
42	5.8±0.17[b]	6.2±0.29[b]

1) Data expressed as mean ± standard deviation n= 15.

2) Data followed by different superscripts are significantly different with p< 0.5.

Figure 3.1. *S*. Infantis caecal colonization of broilers with and without monensin



3.4.2. TREATMENT WITH SODIUM CHLORATE:

3.4.2.1. CAECAL COLONIZATION

When 10- and 20-day old birds were given sodium chlorate in drinking water, caecal colonization by *S.* Infantis decreased significantly ($P<0.05$) 4 and 7 days after treatment (Table 3.2). In birds given sodium chlorate on day 10 through 14, *S.* Infantis populations were 1.2 and 1.0 logs lower on days 14 and 17, respectively, compared to the untreated controls (Figure 3.2). For the group treated with sodium chlorate on day 20, this difference was even greater with numbers of *S.*Infantis in the caeca being 2.9 and 2.8 logs lower on days 24 and 27, respectively, compared to the control group. In contrast, no significant difference in caecal colonization was seen in birds when sodium chlorate treatment was given for 4 days starting on day 38.

3.4.2.2. ORGAN INVASION

No significant differences in *S.*Infantis isolation rates from liver samples were observed between the three treatment groups and the control group (Table 3.3). Since *S.* Infantis was not detected by direct plating, (< 2.0 log CFU/g), all liver samples were subsequently enriched. When the sodium chlorate treatment was initiated on day 10, 33% of the liver samples were free of *S.*Infantis 4 and 7 days post-treatment. The same results were observed four days after treatment when birds began receiving sodium chlorate on day 20 with 66% of the samples negative 7 days after treatment. For birds receiving sodium chlorate on day 38, all liver samples were positive on day 42, as were the controls.

Table 3.2 Mean *S.* Infantis caecal colonization (log CFU/g) using sodium chlorate.

Age of birds	Control [1]	Sodium chlorate treatment [1]		
		Day 10 [2]	Day 20 [2]	Day 38 [2]
7	7.1±0.3 [a3]	6.3±0.3 [a]	6.2±0.3 [a]	6.6±0.3 [a]
10	6.5±0.3 [a]	6.5±0.3 [a]	NA[4]	NA
14	5.7±0.3[a]	4.5±0.3 [b]	NA	NA
17	5.4±0.3 [a]	4.4±0.4 [b]	NA	NA
20	5.4±0.3 [a]	5.2±0.3 [a]	5.1±0.3 [a]	NA
24	5.9±0.3 [a]	NA	3.0±0.6 [c]	NA
27	5.7±0.3 [a]	NA	2.9±0.4 [c]	NA
30	5.2±0.3 [a]	NA	4.8±0.4 [a]	NA
38	5.6±0.3 [a]	NA	NA	5.8±0.3 [a]
42	5.3±0.4 [a]	6.1±0.3 [a]	6±0.4 [a]	5±0.4 [a]

1) Data expressed as mean ± standard deviation (n=6).

2) Day sodium chlorate treatment was started.

3) Data followed by different superscripts are significantly different with $p < 0.5$.

4) Samples not analyzed at these time points as birds were not sacrificed.

Figure3.2. S. Infantis caecal colonization using sodium chlorate

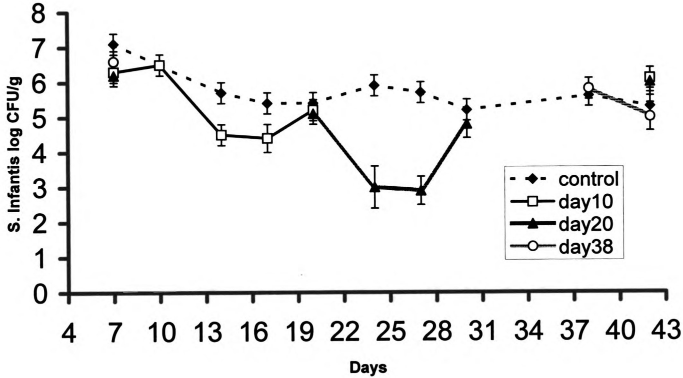


Table 3.3 Invasion of S.Infantis into the liver using sodium chlorate.

Age of birds	Control	Sodium chlorate treatment started		
		Day 10[1]	Day 20[1]	Day 38[1]
7	6 / 6[2]	2 / 6	6 / 6	6 / 6
10	6 / 6	6 / 6	NA	NA
14	5 / 6	4 /6	NA	NA
17	6 / 6	4 / 6	NA	NA
20	6 / 6	6 / 6	6 / 6	NA
24	5 / 6	NA[3]	4 / 6	NA
27	6 / 6	NA	2 / 6	NA
30	6 / 6	NA	6 / 6	NA
38	6 / 6	NA	NA	6 / 6
42	6 / 6	6 / 6	6 / 6	6 / 6

1) Date sodium chlorate treatment was started.

2) Number of positive samples / number of samples.

3) No samples analyzed as birds were not sacrificed at these time points.

3.4.2.3. FECAL SHEDDING:

Fecal shedding of *S.* Infantis was highly irregular. A significant difference ($P < 0.0008$) between the four groups was observed with lower counts in cloacal swabs after treatment with sodium chlorate on days 10 and 20 (Table 3.4). A reduction of > 1.4 and > 0.6 log CFU/g was observed after treatments on days 10 and 20 respectively (Figure 3.3). Although *Salmonella* counts decreased below the level of direct detection (2.0 log CFU / g) on day 13, the levels started increasing on day 15. When treatment was given on day 20, the levels decreased below detectable limit on day 23, as in the previous case with fecal shedding only increasing on day 28. When *S.* Infantis was not detected by direct plating, the enriched samples were invariably positive indicating that low levels of *S.* Infantis were being shed at the above time points.

3.4.2.4. CONFIRMATION OF ISOLATES:

Twenty isolates from caecal, liver or cloacal swab samples in the sodium chlorate study were serotyped at MDCH. Twelve of the 20 isolates were confirmed as *S.* Infantis with the remaining 8 isolates also identical to *S.* Infantis (6,7:-:1,5 or 6,7:r-) except for a phase change in one of the H-antigens (Table 3.5).

Table 3.4 Mean Fecal shedding of *S.* Infantis (log CFU/g) using sodium chlorate.

Age of birds	Control [1]	Sodium chlorate treatment [1]		
		Day 10 [2]	Day 20 [2]	Day 38 [2]
11	4.3±0.38 [a3]	3.2±0.38 [b]		
12	3.1±0.38 [a]	4.4±0.38 [a]		
13	4.3±0.38 [a]	< 2.7 [b4]		
15	4.0±0.38 [a]	2.8±0.38 [b]		
16	3.7±0.38 [a]	4.0±0.38 [a]		
18	4.0±0.38 [a]	3.2±0.38 [a]		
19	3.0±0.38 [a]	3.8±0.38 [a]		
21	3.2±0.38 [a]		3.3±0.38 [a]	
22	4.0±0.38 [a]		3.2±0.38 [a]	
23	4.0±0.38 [a]		< 2.7 [b4]	
25	4.0±0.38 [a]		< 2.7 [b4]	
26	4.1±0.38 [a]		< 2.7 [b4]	
28	3.2±0.38 [a]		2.8±0.38 [a]	
29	3.6±0.38 [a]		< 2.7 [b4]	
39	3.3±0.38 [a]			3.2±0.38 [a]
40	3.0±0.38 [a]			2.9±0.38 [a]
41	3.8±0.38 [a]			2.9±0.38 [a]

1) Data expressed as mean ± standard deviation (n=6).

2) Day sodium chlorate treatment started.

3) Data followed by different superscripts are significantly different with $p< 0.5$.

4) *S.* Infantis only detected post-enrichment and these were given a value of log 2.7 for statistical purposes.

S. Infantis fecal shedding using sodium chlorate

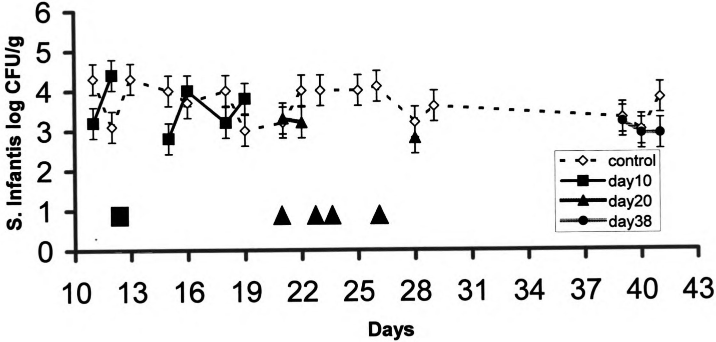


Table 3.5. Confirmation results on *S.* Infantis selected isolates

MDCH Lab #	MSU Lab #	Serotype
1	D-15-F(10)	Infantis
2	D-30-Liv (C)	Infantis
3	D-17-F(10)	Infantis
4	D-28-F(20)	Infantis
5	D-42-Ce(20)	Infantis
6	D-42-E-g&p(31-33)	Infantis
7	D-14(10)	Infantis
8	D-42-Ce(38)	6,7:-:1,5
9	D-41-F(38)	6,7:-:1,5
10	D-40-F(38)	6,7:-:1,5
11	D-25-Ce(C)	6,7:-:1,5
12	D-30-Ce(20)	Infantis
13	D-29-F(C)	Infantis
14	D-21-F(20)	6,7:r:-
15	D-17-F(C)	Infantis
16	D-24-Liv(C)	Infantis
17	D-20-Liv(C)	6,7:r:-
18	D-19-F(10)	6,7:-:1,5
19	D-13-F(C)	6,7:r:-
20	D-24-E-Ce(20)	Infantis

3.4.2.5. BODY WEIGHTS:

A significant difference ($P < 0.0001$) in bird body weight was observed between the groups (Table 3.5). Birds treated with sodium chlorate on day 10 and 20 as a group had greater weight gain when the study concluded on day 42. Weight was gained during the period after treatment when the birds had decreased caecal populations of *Salmonella*. However, weights of birds treated on day 38 were similar to the controls. No significant differences between bird body weights in groups were observed.

Table 3.5. Body weights of broilers using sodium chlorate as recorded on day 42.

Birds	Weight in pounds			
	Control	Sodium chlorate started on		
		Day 10	Day 20	Day 38
1	0.25 ^{a}	0.875 ^{b}	0.5 ^{b}	0.25 ^{a}
2	0.25 ^{a}	0.8125 ^{b}	0.875 ^{b}	0.375 ^{a}
3	0.375 ^{a}	1.0 ^{b}	0.875 ^{b}	0.25 ^{a}
4	0.25 ^{a}	1.0 ^{b}	0.875 ^{b}	0.25 ^{a}
5	0.4375 ^{a}	0.8125 ^{b}	1.0625 ^{b}	0.3125 ^{a}
6	0.25 ^{a}	1.0625 ^{b}	0.8125 ^{b}	0.25 ^{a}

Data followed by different superscripts are significantly different.

3.5. DISCUSSION:

No difference in caecal colonization of *S.* Infantis was observed when broilers were given feed containing monensin or an antimicrobial-free feed, with *Salmonella* populations only decreasing about 1 log during the lifetime of the birds. Hence, the ionophore antibiotic monensin had no effect on *S.*Infantis colonization of the caeca in broilers.

Relatively few reports concerning the effect of monensin on *Salmonella* have appeared in the literature since monensin is primarily used as a coccidiostat in the poultry industry. However, when broilers were fed avoparcin (10 ppm) and monensin (90 ppm), Holmberg et al., (1984) did report higher caecal levels of *S.*Infantis in comparison to feeding only avoparcin during a 2-week challenge period. A significant increase ($p<0.5$) in numbers of *Salmonella* in caeca 7, 10 and 14 days after coccidia infection was observed by Arakawa et al. (1981), indicating that treatment with an anticocidiostat would decrease salmonellae. Work by Morishima et al. (1984) also suggests that *Salmonella* counts can increase when chickens are simultaneously infected with coccidia.

Many studies have dealt with the effect of monesin on coccidia (Chapman and Saleh, 1999; Hooge et al., 1999). When fed at a rate of 59.5 ppm, monensin reportedly reduced mortality and improved feed conversion, leading to increased weight gain in turkey poults (Chapman and Saleh, 1999). Monensin in combination with sodium bicarbonate was also found to significantly reduce mortality due to coccidia in broilers (Hooge et al., 1999). In our study which did not assess the impact of coccidia, failure of monensin to decrease colonization by *Salmonella* might be due to the continuous exposure of our broilers to high levels of *Salmonella* in the environment.

Treatment with sodium chlorate reduced caecal colonization of *Salmonella* in broilers by up to 2 logs, with birds receiving sodium chlorate on days 10 and 20 having significantly lower levels of *S.*Infantis in the caeca, when compared to the untreated control birds. These findings confirm the bactericidal effectiveness of sodium chlorate on *Salmonella* in birds as previously reported by Anderson et al. (2000) in *in vitro* studies with rumen contents. *Salmonella* possess respiratory nitrate reductase activity, which is responsible for the anaerobic reduction of nitrate to nitrite. This same enzyme will also catalyze the intracellular reduction of chlorate to cytotoxic chlorite with this end product being lethal to *Salmonella* and other bacteria such as *Escherichia coli* O157:H7 that possess nitrate reductase activity. An added advantage of sodium chlorate is that the normal gastrointestinal flora is not affected (Anderson et al., 2000).

Despite these reports, we found sodium chlorate to be ineffective when given to 38-day old birds that were sacrificed 4 days later. The lack of any reduction of *S.* Infantis in the caeca of 38- to 42-day old birds may be due to the establishment and spread of *S.* Infantis from the gastrointestinal tract to the internal organs since these birds were showing overt signs of salmonellosis as evidenced by reduced feed intake, droopiness, greenish diarrhea and a high mortality rate. Also the fact that these birds were raised in an environment with high levels of *Salmonella* may have decreased the effectiveness of the treatment with reinfection of the gastrointestinal tract occurring in those birds which had decreased levels of *Salmonella* after treatment on days 10 and 20.

S. Infantis reductions of 1 and 2 logs in the caeca were observed in birds given sodium chlorate beginning on days 10 and 20, respectively. These findings agree those of Anderson et al., (2001) who treated pigs with sodium chlorate. Caecal levels of *Salmonella* were found to decrease four days after treatment in both of the above groups, with this decrease evident up to seven days post-treatment in birds treated on day 20. However, in our study populations of *S.* Infantis began increasing 7 days post-treatment due to continuous environmental exposure associated with fecal shedding of the organism.

In contrast to our favorable results for cecal colonization, sodium chlorate failed to prevent liver invasion by *S.* Infantis. Although all liver samples were negative by direct plating, most liver samples were positive on enrichment. These findings are similar to those observed by Brown et al., (1976) who also reported frequent recovery of *S.* Infantis from the liver as well as the lungs and spleen of cockerals. A lower concentration of the organism in liver might result from the fact that the caecum is the main colonization site for *Salmonella* (Hinton et al., 1990). Although frequently positive for *S.* Infantis, cloacal swab samples showed wide variability within treatment groups. Intermittent shedding of *S.* Infantis was also observed by Brown et al., (1976) in a study wherein birds were orally inoculated with the organism into the middle third of the esophagus.

In our work, feeding of sodium chlorate significantly decreased fecal shedding of *S.* Infantis with counts decreasing > 0.6 to > 1.4 logs / g after treatment on days 10 and 20. Hence, four-day long sodium chlorate treatment reduced both caecal colonization and fecal shedding. When the birds were treated with sodium chlorate on day 20, *S.* Infantis colonization decreased in young broilers (24 days of age) with sodium chlorate remaining effective for up to 7 days after treatment. Thus, these findings suggest that sodium chlorate may be helpful for reducing *Salmonella* colonization in incoming birds and thereby decreasing carcass contamination during slaughter and further processing. In conjunction with the USDA-FSIS mandated HACCP programs, sodium chlorate treatment of broilers may help processing plants meet and/or exceed the *Salmonella* performance standards established by the USDA baseline study.

CHAPTER 4

CONCLUSIONS AND FUTURE RESEARCH

Salmonella colonization in the gastrointestinal tract of broilers leads to cross-contamination of poultry carcasses, thus increasing the number of foodborne salmonellosis outbreaks. According to the Pathogen Reduction / Hazard Analysis and Critical Control Point final rule issued by FSIS-USDA, all poultry processing plants are required to meet the *Salmonella* performance standards which have been fixed at 20%. Control of *Salmonella* in integrated poultry operations is often very difficult as there are numerous sources of *Salmonella* in the poultry industry such as the feed, water, flies and other insects, farm environment and processing environment. Thus, the poultry industry is always seeking easier, more effective and more economical ways to control *Salmonella*.

The primary source of *Salmonella* contamination in poultry processing plants is the gastrointestinal tract of broilers. In the present study, we evaluated two different treatments for reducing / eliminating *Salmonella* colonization in the intestinal tracts of broilers. The first treatment, in which broilers received feed supplemented with monensin, did not provide a method to control *Salmonella* in the gastrointestinal tract. These findings were not entirely unexpected since monensin is used primarily as a coccidiostat in the poultry industry. In contrast, treatment of broilers with sodium chlorate showed some promise for decreasing the incidence of *S.*Infantis in broilers. Caecal colonization of *Salmonella* decreased when broilers received sodium chlorate in their drinking water over a period of four days.

Our present study only evaluated the effects of sodium chlorate and monensin on *S.* Infantis in broilers. The effectiveness of sodium chlorate in poultry needs to be further assessed against a wider variety of *Salmonella* species in poultry. Future studies should examine the effect of sodium chlorate on other strains of *Salmonella* particularly *Salmonella* Typhimurium DT 104 and other multi-antibiotic strains. Efficacy of sodium chlorate as a terminal treatment in broilers, the exact dose of sodium chlorate required to eliminate *Salmonella* from the gastrointestinal tract of birds and field trials using sodium chlorate as a preventive measure are also worth investigating. Key insights into the reduction of poultry-borne diseases could be gained by examining the effectiveness of sodium chlorate against other poultry-borne pathogens such as *Escherichia coli.* Other areas of research could also include the effect of long term treatment with sodium chlorate as well as the effect of sodium chlorate on the end product in terms of safety. The outcome of further investigations will help in coming to a definite conclusion as to the effectiveness of the current method.

APPENDIX

School Project:

Broiler chicks raised by FFA (Future Farmers of America) students in different schools in Michigan State were processed at the Fowlerville processing unit on the 1st and 2nd of November, 2001. Samples were collected from 25 of 39 schools participating. Caecal samples were taken from five birds at random from each school. Sample collection was done as aseptically as possible in the processing unit. Caecal samples were collected in individual stomacher bags, transported from the processing unit to the laboratory in ice boxes and analyzed immediately. Each sample was tested for the presence of *Listeria* and *Salmonella* using the REVEAL microbial screening test for *Salmonella* and *Listeria* (Neogen Corporation, Lansing, MI). Of 75 birds tested, 57 and 56 were positive for *Salmonella* and *Listeria* respectively. The positive REVEAL enrichment cultures for *Salmonella* were plated on Hektoen enteric agar, Bismuth sulfite agar and Xylose lysine desoxycholate agar for verification. After plating, only 49 cultures were found positive for *Salmonella*. Similarly, *Listeria*-positive REVEAL enrichment cultures were plated on Oxford agar and Trypticase soy yeast extract agar (Difco) for verification with only 48 samples positive for *Listeria*. Picking and inoculating two colonies per plate into Triple sugar iron and Lysine iron agar (Difco) slants was used to presumptively confirm *Salmonella* isolates. These isolates which were then grown in Tryticase soy broth, were frozen down to –80°C.

Table: List of schools positive for Salmonella and Listeria

Number	Name of school	No. of samples positive for *Salmonella*	No. of samples positive for *Listeria*
1	North Adams-Jerome	-	5
2	Dansville	5	5
3	Olivet	4	5
4	Akron-Fairgrove	4	3
5	Springport	5	-
6	Durand	3	1
7	Alpena	1	2
8	Milan	3	1
9	Dundee	1	4
10	Ubly	2	3
11	Vicksburg	-	-
12	Bronson	-	2
13	Beal City	2	5
14	Roosevelt	2	-
15	USA	1	-
16	Byron	3	-
17	River Valley	-	-
18	Alma	2	3
19	Waldron	1	1

20	Branch ACC	2	1
21	Ionia	2	2
22	Laingsburg	1	1
23	Port Hope	2	1
24	Chesaning	2	2
25	Corunna	1	1

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