EFFICACY OF FEEDING TREATMENTS AND LITTER FORMULATIONS AGAINST SHEDDING OF *SALMONELLA* IN BROILERS

A Thesis

by

ERYN LINNAE LARRISON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	Michael Davis
Committee Members,	John Carey
	David Caldwell
Head of Department,	John Carey

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ABSTRACT

Efficacy of Feeding Treatments and Litter Formulations against Shedding of *Salmonella* in Broilers. (May 2009)

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Chair of Advisory Committee: Dr. Michael Davis

Research has shown that *Salmonella* can be prevalent in poultry litter, which can be a source of contamination for newly arrived chicks at the poultry house. Since this organism is a pathogen of concern to the poultry industry, two types of litter amendments and two types of feed additions were created and tested to determine effects of broiler growth, litter moisture and efficacy against *Salmonella* colonization. Litter amendments consisted of the combination of acidic calcium sulfate (ACS) with either diatomaceous earth (DE) or hydrated sodium calcium aluminiosilicate (HSCAS). Feed additions consisted of differing amounts of sodium bisulfate.

For the litter amendment experiments, chicks were placed into pens in isolation rooms. Each litter amendment was applied to 3 pens for replicates of experimental groups. Litter samples were taken weekly from 5 areas in each pen and combined for the determination of *Salmonella* cfu/g of litter. At 3 and 6 weeks post placement, birds from each pen were euthanized by CO_2 asphyxiation. The crop and ceca from these birds were tested for *Salmonella* cfu/g of crop/ceca or presence/absence of *Salmonella*.

Efficacy of the liter amendments varied in experimental groups on broiler growth characteristics and efficacy against *Salmonella*.

For the determination of sodium bisulfate as a feed addition on *Salmonella* colonization, birds were placed into 3 experimental groups and placed into respective pens. Two sodium bisulfate experimental groups were used at 0.25% and 0.75%. The other experimental group was the control. Litter samples were taken weekly from 5 areas in each pen and combined for the determination of *Salmonella* cfu/g of litter, aerobic plate counts, moisture content and pH. Efficacy of the sodium bisulfate as a feed addition varied in the experimental groups on *Salmonella* colonization, aerobic plate counts, pH and moisture content throughout both trials.

DEDICATION

I would like to dedicate my work to my mother, father and grandparents. You all have guided and supported me this far in life and I know that you all will be there for me in the future. Mom and dad, you have always allowed me to reach for the stars and I can only hope to be half as good as a parent to my children as you all have been to me. I love you and thank you.

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I would like to thank my committee chair, Dr. Davis, and my committee members, Dr. Carey and Dr. Caldwell, for their leadership and direction over the course of my research and overall education. You all have always been there to answer any questions and help guide me in the right direction. Thank you again for everything.

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CHAPTER I

INTRODUCTION

Paratyphoid *Salmonella* is a major human foodborne pathogen of concern which is associated with poultry and poultry products. Contamination of poultry products by paratyphoid *Salmonella* has increasingly contributed to significant human foodborne illness, with an estimated annual incidence of 1 million to 4 million cases in the United States (Tauxe, 1996). The government and poultry industry have been implementing programs to reduce contamination. One such program is HACCP (Hazard Analysis Critical Control Points) (FSIS, 1996). This program requires poultry processors to comply with *Salmonella* performance standards set by the USDA. Each plant has its own HACCP plan which if followed will help to produce a safer product and be able to prove it. Hazards, including physical, chemical and biological are evaluated and preventative measures are the put into place. This program has proven to be effective in reducing hazards in processing facilities.

Since paratyphoid *Salmonella* foodborne illness outbreaks are on the rise as well as an increase in the consumption of poultry products, research in this area is increasing. Poultry product contamination poses both human and financial risks (Hayes, 2000). Loss of consumer confidence as a result of outbreaks has also been of concern to the poultry industry (Hayes, 2000). HACCP success cannot solve all food safety

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This thesis follows the style of Poultry Science.

concerns alone. This is why further investigations and studies into alternative intervention strategies to reduce microbial loads are necessary for the control of *Salmonella* in the poultry industry.

HACCP is a processing facility control program. The birds entering the plant are already contaminated with my different microorganisms and pathogens. Controlling these pathogens at the farm level is the next step to reducing pathogens entering the processing plant and eventually leaving on the final product. Interventions to control microbial populations on growout farms include litter amendments or feed additions. The present research in this thesis focuses on the efficacy of two different types of litter amendments and feeding sodium bisulfate in differing levels to reduce *Salmonella* contamination in broiler litter.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Food safety is a subject of ever growing concern for the citizens in the United States. The contamination of broiler carcasses with human enteropathogens remains a problem of the broiler industry, food safety regulatory agencies, and concerned consumers (Corrier et al., 1999). Paratyphoid *Salmonella* continues to be a foodborne pathogen of concern and has been linked to the consumption of contaminated poultry products (Tauxe, 1991; Bryan and Doyle, 1995; Hoszowski et al., 1996; Byrd et al., 1997; Cox et al., 2000). *Salmonella* is often present in the intestinal tracts of mammals and birds, is readily acquired from feed and environmental sources, and contaminates body parts of fowl on the farm (Bryan and Doyle, 1995). It has also been found that *Salmonella* contamination of poultry carcasses is believed to occur during processing and originates from the feathers, skin surface, and intestinal contents of the birds (Simmons and Byrnes, 1972), as well as from transport crates (McGarr et al., 1980; Rigby et al., 1980) and the plant environment (McGarr et al., 1980). The farm environment may also be contaminated (Renwick et al, 1992).

Paratyphoid *Salmonella* is a pathogen of concern due to the fact that isolation of this organism from humans in the U.S. has risen linearly since 1955 and a fourfold increase in reported isolations had accrued by the 1990s (Center for Disease Control, 1992). Contamination of poultry products by paratyphoid *Salmonella* has increasingly

contributed to significant human foodborne illness, with an estimated annual incidence of 1-4 million cases in the United States (Tauxe, 1996). This pathogen not only has the potential to cause disease in humans, but because of increased availability of data concerning cases and outbreaks, consumers have access to more information which can lead to lost consumer confidence in branded products along with lost confidence in safety of poultry products in general.

Salmonella the Organism

The genus *Salmonella* is within the genera *Enterobacteriacea*. This family includes other well known pathogens such as *Escherichia* and *Shigella*. The microorganism, *Salmonella,* is a small gram-negative rod that is non-sporeforming and is facultatively anaerobic. This microorganism is generally motile by peritrichous flagella and is considered to be mesophilic and grow best at body temperatures of host organisms. This pathogen is able to survive highly acidic environments, but cannot grow in these conditions.

Salmonella are widely distributed in nature, with humans and animals being their primary reservoirs (Jay et al., 2005). The genus *Salmonella* contains over 2,000 serotypes that have been classified by somatic (O), flagellar (H), and capsular (Vi) antigens (Porter, 1998). All salmonellae have been placed in two species, *S. enterica* and *S. bongori*, with the 2,000 or so serovars being divided into five subspecies or groups (Jay et al., 2005).

For epidemiological purposes, the salmonellae can be placed into three groups:

1. Those that infect humans only: These include *S.* Typhi, *S.* Paratyphi A, and *S.* Paratyphi C (Jay et al., 2005). This group includes the agents of typhoid and paratyphoid fevers, which are the most severe of all diseases caused by salmonellae (Jay et al., 2005). Typhoid and paratyphoid fever, which are rare in developed countries, are transmitted human to human by the fecal-oral route, and humans are the only reservoir (Sams, 2001).
2. Host-adapted serovars: Included are *S. Gallinarum* (poultry), *S. Dublin* (cattle), *S.* Abortus-equi (horses), *S.* Abortus-ovis (sheep), and *S. Choleraesuis* (swine) (Jay et al., 2005).
3. Unadapted serovars or paratyphoid salmonellae (no host preference): these are pathogenic for humans and other animals, and they include most foodborne serovars (Jay et al., 2005). Gastroenteritis (non-typhoidal salmonellosis) is caused by *Salmonella enterica* serotypes, which are found in the intestinal tract of both humans and non-human animals (Sams, 2001). Poultry has been identified as a primary reservoir for these salmonellae (Bryan and Doyle, 1995).

The primary habitat of *Salmonella* spp. is the intestinal tract of animals such as birds, reptiles, farm animals, humans, and occasionally insects (Jay et al., 2005). It has been noted that *Salmonella* is carried in animal's intestinal tract asymptomatically; however, they can be shed in feces in large populations and be transmitted by other vectors from feces to animals, produce, or humans (Doyle and Erickson, 2006). As intestinal forms, they may also be found in water, especially polluted water (Jay et al., 2005). The previously mentioned vectors illustrate how *Salmonella* can be transmitted horizontally.

Horizontal transmission is when a pathogen is transmitted within a population. *Salmonella* is also capable of vertical transmission from the hen to her progeny through the egg laying process.

Optimum growth conditions for salmonellae include a pH around neutral, with values above 9.0 and below 4.0 being bactericidal (Jay et al., 2005). It has been found that the best pH for growth is between 6.6 and 8.2 (Jay et al., 2005). According to Jay and colleagues, 45^{o}C has been found to be the upper limit for growth with the optimum temperature being around 37^{o}C. *Salmonella* spp. are not salt tolerant. Brine above 9% is reported to be bactericidal (Jay et al., 2005). It should also be noted that *Salmonella* is not heat resistant. Normal pasteurization temperatures eliminate the microorganism from eggs and milk products.

Salmonella **in Chickens**

Of the host adapted *Salmonella* serotypes, *S. Pullorum* and *S. Gallinarum* have been associated with avian species. *S. Pullorum* causes pullorum disease (PD) in chickens and *S. Gallinarum* is responsible for fowl typhoid (FT) (Kwon et al., 2000). Fowl typhoid can be extremely pathogenic in young chickens with mortality being well over 50 percent (Lowry et al., 1999). In the U.S., breeding flocks are serologically monitored for pullorum-typhoid through the National Poultry Improvement Plan and there is mandatory disease reporting and quarantine of affected flocks (USDA Animal Health Inspection Service, 1994). Such programs have drastically reduced the incidence of pullorum-typhoid disease in the U.S. (Porter, 1998).

Mortality due to pullorum disease is usually observed in chicks ≤ 3 weeks old. Pullorum disease occurs throughout the world but is rare in the United States commercial settings. This disease has not been eradicated in other countries throughout the world. Clinical signs of pullorum disease in chickens include grayish-white necrotic foci in lungs with possible similar lesions in the heart and liver (Randall, 1985). Other signs include synovitis of hock joints in chicks and involvement of the ovary in sexually mature hens (Randall, 1985). The best control for pullorum disease is prevention. Pullorum-free breeding flocks should be established and maintained according to the National Poultry Improvement Plan (NPIP).

S. Gallinarum causes fowl typhoid which mainly affects chickens but it can affect other avian species as well. The infection caused by this microorganism still causes problems worldwide but is rare in the commercial poultry setting in the United States. Fowl typhoid can cause mortality in any age bird but it is usually observed in young chicks. In most acute cases the lungs show a brown discoloration (Randall, 1985). The carcasses of birds that have died from the acute disease are jaundiced and the liver shows a characteristic bronzing after exposure to the air (Randall, 1985). As with pullorum, prevention is the best control measure for fowl typhoid.

Birds that recover from PD and FT can become chronic carriers and transmit the infection to progeny through the eggs (transovarian transmission) (Porter, 1998). Infected progeny can readily spread the infection horizontally through contaminated feces (Porter, 1998). Adult birds from flocks affected by PD or FT normally become carriers and show no clinical signs of the disease.

The paratyphoid *Salmonella* group is known to infect a wide variety of host species including poultry. The paratyphoid group of *Salmonella* does not necessarily cause illness to the species but has been observed. The intestinal colonization by paratyphoid *Salmonella* may result in invasion of the gut wall and dissemination to internal organs (Brown et al., 1976). Paratyphoid infections in young birds often result in systemic infections with high mortality (Porter, 1998). Adult birds appear to be rather resistant to paratyphoid infections but may harbor *Salmonella* in soft tissues without showing clinical signs (Brown et al., 1976). Adult hens that are infected with *S. Enteritidis* (SE) will appear healthy and continue to shed SE in the feces (Holt and Porter, 1993). It should be noted that the paratyphoid group of salmonellae is a major concern to the poultry and food industry because they can cause foodborne illness in humans and many are zoonotic.

Horizontal transmission of paratyphoid salmonellae is through the oral route, while vertical transmission can be accomplished through shell contamination. Many species are considered to be carriers of different serotypes of *Salmonella.* These animals carry the microorganism in their intestines and shed them over time. This allows for transmission of these bacteria through feces. Rodents are especially a problem for farms due to the fact that many are intestinal carriers.

Clinical signs of paratyphoid infections in chickens are generally mild in nature compared to other host specific salmonellae or they may be entirely absent. Biosecurity and proper sanitation are very important to avoid and control contact with paratyphoid *Salmonella*. Paratyphoid *Salmonella* and other bacteria are often found in the

environment but are usually vulnerable to commercially available disinfectants. Removing rodents and pests from premises where birds are housed are essential to keep and maintain paratyphoid *Salmonella* free houses and birds.

Numerous factors can affect the susceptibility of chickens to salmonellae colonization, including age, stress, general health, feed additives, the genetics of the chicken, and others (Bailey, 1988). It has been reported that newly hatched chicks are more susceptible to salmonellae colonization than older chicks that have developed resistance as native microflora become established (Kubena et al., 2001). This is one reason why the growth of desirable bacteria in the lower gastrointestinal tract of the chicken has been promoted as a mechanism for reducing undesirable pathogenic and putrefactive bacteria (Chambers et al., 1997).

The ceca have been identified as the primary site of *Salmonella* colonization in poultry (Fanelli et al., 1971; Snoeyenbos et al., 1982). Therefore, cecal and intestinal contents have been considered to be the primary source of *Salmonella* contamination of rearing house floor litter, the external surfaces and feathers of broilers, and of processed carcasses after rupture of the intestinal tract during evisceration in processing facilities (Corrier et al., 1999). Paratyphiod *Salmonella* contamination of broiler carcasses continues to be a potential problem for the broiler industry (Ramirez et al., 1997). Cross contamination has been shown to increase with successive stages of processing (Lillard, 1989). Poultry integrators have and continue to develop on-farm strategies to control paratyphoid *Salmonella* with the desired result of decreasing initial populations of the organism entering the processing environment.

Pathogen carriage in livestock and poultry leads to both direct and indirect contamination of food products (Doyle and Erickson, 2006). The main components of contamination of poultry include the feathers and skin. It is also known that water is an important vehicle for enteric pathogen dissemination (Doyle and Erickson, 2006). Primm (1998) found that finished feeds represented an important source of salmonellae contamination in commercial turkey flocks. Analyses of commercially manufactured feeds confirmed that both feed ingredients and dust can be sources of *Salmonella* contamination in feed mills (Jones and Richardson, 2004). According to Jones and colleagues (1991) study of *Salmonella* contamination in modern broiler production, *Salmonella* was most frequently found in samples collected from feed mills. Results from this study found that *Salmonella* was isolated from mash feeds at a rate of 35% and from pelleted feeds at a rate of 6.3% (Jones et al., 1991). The predominant serotype that was isolated from pelleted feeds was *Salmonella* Typhimurium (Jones et al., 1991). Their data also indicated that pelleting reduces *Salmonella* isolation rates in feeds by 82.0% and 81.1% respectively (Jones et al., 1991). Thus, the pelleting process is effective at reducing isolations from feeds but does not completely eliminate these bacteria (Jones et al., 1991). In contrast, Bailey and colleagues (2001) research concluded that feed contamination may not be a cause of carcass contamination. Their research was able to identify 10 different serotypes of *Salmonella* from feed samples; however, on only one occasion was the same serotype found in the feed also found on the final processed carcass (Bailey et al., 2001).

Wildlife and pests also can be sources of pathogens on the farm (Doyle and Erickson, 2006). *Salmonella* was isolated from Lesser meal worms, American cockroaches, and German cockroaches in Jones and colleagues (1991) study of *Salmonella* contamination. Ten years later, Bailey and colleagues (2001) were able to recover *Salmonella* from flies (18.7%). They found this to be very interesting and may suggest that flies may be an inexpensive and easy sample to screen houses and flocks for the presence of this microorganism (Bailey et al., 2001). Animal housing and transportation equipment can also harbor pathogens and contribute to contamination of animals; however, a primary source of enteric pathogens transmitted to livestock and poultry is manure (Doyle and Erickson, 2006).

Since poultry manure has been identified as a primary source of enteric pathogens such as paratyphoid *Salmonella*, poultry litter has been studied. The sampling of poultry house litter has been used to indicate the *Salmonella* status of broiler flocks for the past 25 years (Kingston, 1981). It has been suggested that when the chickens peck at infected litter it gives *Salmonella* a chance to enter the intestinal tract of the bird and continue to cycle throughout the house and other birds through the fecal-oral route of transmission.

Salmonella **in Humans**

Salmonella food poisoning results from the ingestion of foods containing appropriate strains of this genus in significant numbers (Jay et al., 2005). The disease caused by this organism is commonly referred to as salmonellosis. Paratyphiod

Salmonella's frequency of involvement in foodborne disease is considered to be very common (ICMSF, 2002). There are three different types of salmonellosis in humans which are caused by different strains of paratyphoid *Salmonella*. The types of salmonellosis include typhoid fever, enteric fever and gastroenteritis syndromes.

In general, from the time of ingestion of the organism, symptoms usually develop in 24-48 hours, although shorter and longer times have been reported (Jay et al., 2005). The symptoms consist of nausea, vomiting, abdominal pain, headache, chills, and diarrhea (Jay et al., 2005). These symptoms usually persist for 2-3 days. Although the organism generally disappears rapidly from the intestinal tract, up to 5% of patients may become asymptomatic carriers of the organisms upon recovery from this disease (Jay et al., 2005). Carriers have the potential to distribute the organism to other humans as well as the environment. Numbers of cells on the order of 10^7-10^9/g are generally necessary for salmonellosis (Jay et al., 2005). Lower numbers have been observed to cause sickness in rare cases.

Typhoid fever is caused by *Salmonella typhi* and is the most severe form of salmonellosis. Since humans are the only known reservoir for *S. typhi*, contamination by direct contact from an infected human is the only way to transmit the disease. Contamination usually occurs by ingesting food from a food handler who has been exposed to *S. typhi*. An example of this process is Mary Mallon, who was known as Typhoid Mary. She was a cook in the 1900s in New York and also an asymptomatic carrier of *S. typhi*. Outbreaks occurred throughout the city and were linked back to her.

She eventually was imprisoned since she would not comply with court orders to stop preparing food in commercial establishments.

Symptoms of typhoid fever include the general symptoms of salmonellosis but also septicemia and bleeding from the bowel. These symptoms can last anywhere from one to eight weeks.

Contaminated food and water are two main vehicles for the transmission of *S. typhi.* Since humans are the reservoir for this microorganism, infected food handlers are also considered to a vehicle of transmission. Foods that are not properly heat treated (cooked) and subsequently handled by an infected person have been a found to be a main source of transmission. Also, foods that have been cooked properly then cooled and handled again cause cross contamination and can transmit the microorganism.

Enteric fever is caused by any of the three types of *Salmonella paratyphi.* This form of salmonellosis is very rare in the United States. Symptoms of enteric fever are similar to typhoid fever but are less serve. The duration of this disease normally lasts anywhere from one to three weeks. Sources of *S. paratyphi* include many raw products such as raw milk, raw eggs and raw salads.

Gastroenteritis syndrome is another type of salmonellosis. This syndrome is more commonly known as food poisoning. Gastroenteritis is caused by all other types of *Salmonella.* This is the most common form of salmonellosis found in the United States.

Symptoms include the general symptoms associated with salmonellosis. The severity of the disease depends on the host's immune system. It has been observed that most deaths occur in the elderly and the young due to a reduced immune response.

Death may also occur through dehydration. The incubation period of gastroenteritis is usually 12-36 hours with a duration time of one to four days.

The best medical treatment for gastroenteric food poisoning is to nothing since most forms are self limiting. If a clinical case is encountered, seek medical attention where antibiotics will most likely be administered. Remember to take the antibiotics as prescribed for the allotted time frame.

There are many routes of transmission for *Salmonella* when discussing food poisoning. The principal source of infection and most common way is by ingestion of contaminated food. Raw and undercooked poultry and poultry products have been identified as a main vehicle of transmission. According to the CDC (2000), consumption of egg products was identified as a major food vehicle. Another transmission route includes person to person contact. This can be avoided through proper sanitation and personal hygiene. An additional route includes animal to person contact. It is well known that many reptile species are carriers of *Salmonella.*

The precise incidence of salmonellae food poisoning in the United States is not known (Jay et al., 2005). In the largest outbreak of salmonellosis the vehicle food was ice cream produced from milk that was transported in tanker trucks that had previously hauled liquid eggs (Jay et al., 2005). The salmonellae serotype that caused this outbreak was *S.* Enteritidis, which is commonly associated with eggs and egg products. Another large outbreak occurred with an uncommon food vehicle. The vehicle food was potato salad served to about 11,000 individuals at a barbecue (Jay et al., 2005). It was prepared and stored for up to 16 hours at improper holding temperatures prior to serving; the

serovar isolated was *S.* Newport (Jay et al., 2005). A more recent outbreak occurred with peanut butter and peanut butter containing products in 2008 and early 2009. There were 529 lab confirmed cases of *S.* Typhimurium according to the CDC (2009). The CDC (2008) also confirmed 401 cases of paratyphoid *Salmonella* foodborne illness due to frozen pot pies. Of the 401 confirmed cases, 92% of the individuals were able to link the sickness to the specific brand of Banquet (CDC, 2008). The final conclusion concerning this outbreak was consumer confusion regarding microwaving instructions might have resulted in a failure to cook the product properly (CDC, 2008). It may be noted that *S.* Typhimurium has been the single most frequently isolated serotype since about 1975 with *S.* Enteritidis being the second most but this is changing (Jay et al., 2005). According to the CDC (2008), of the 6,299 *Salmonella* isolates serotyped, seven serotypes accounted for 61.6% of infections: Enteritidis, 1,062 (16.9%); Typhimurium, 1,006 (16.0%); Newport, 656 (10.4%) accounted for the highest percentages. Except for the association of *S.* Enteritidis with poultry and egg products, it is difficult to predict the association of most other salmonellae serovars with specific food products (Jay et al., 2005).

Salmonellosis outbreaks and cases continue to increase. One reason for this increase is due to mass food preparation for events such as weddings, conferences, etc. This allows for more people to potentially get sick from one contaminated food source such as the potato salad. When mass amounts of food are prepared, there is a greater chance for improper food storage, temperature abuse, and cross contamination. Temperature abuse or unsuitable storage is also a reason for the increase in

salmonellosis. International trade of produce has been a contributing factor for outbreaks. A relatively recent example traced *S.* Poona back to cantaloupes from Mexico which caused illness in the United States (Jay et al., 2005).

The prevention and control of *Salmonella* includes three factors which are considered to be major players in aiding salmonellosis outbreaks.

- Temperature abuse after cooking
  - Cooking a food product properly will kill any *Salmonella* that may have been on the product but when the cooked product is not stabilized properly, cross contamination (see below) can occur and allow for proliferation of the organism.
- Improper cooking
  - If the product is not heated to specified temperatures, *Salmonella* will not be eliminated from the product.
- Cross contamination
  - This is major source of *Salmonella* contamination. Use one utensil for raw products and another one for cooked products. Another potential site for cross contamination is cutting boards. Cooks place raw meat on a cutting board to prepare it for cooking. While the meat is cooking many prepare a salad and slice vegetables on the same cutting board allowing the salad to be contaminated with the microorganisms from the meat.

Other prevention and control strategies are in place at processing and growout facilities. These will be discussed in more detail in the next section.

Controlling *Salmonella* in Broiler Houses

To successfully meet federal and processing plant pathogen-control standards, recent interest has centered on the implementation of on-farm pathogen reduction programs to reduce contamination loads in and on birds entering the processing plant (Payne et al., 2007). This idea centers around the belief that a reduced numbers of bacteria coming in the plant will allow fewer bacteria to leave the plant on the final product. From a Hazard Analysis Critical Control Point (HACCP) approach, elimination of paratyphoid *Salmonella* from chickens prior to their arrival at the plant would prevent much of the subsequent carcass contamination due to either surface contamination of the live chicken or to crop colonization (Chambers et al., 1998). Culturing of floor litter for the presence of paratyphoid *Salmonella* may be of value in minimizing contamination at slaughter (Long et al., 1980). Houses that are deemed positive for paratyphoid *Salmonella* could then be processed at the end of the processing shift to aid in reducing further contamination. Many researchers have been working to help reduce paratyphoid *Salmonella* in broiler houses.

In order to reduce paratyphoid *Salmonella* contamination of poultry and poultry products, the sources of such contamination must first be identified, so that appropriate measures for control may be developed and implemented (Rigby et al., 1980). Many

different variables have been identified as sources of paratyphoid *Salmonella* contamination such as feed, water, litter, transportation crates and newly hatched chicks.

Many different treatments have been developed and tested throughout the years to try to reduce pathogens on the farm. One such practice included the consumption of 15m*M* of chlorate-treated drinking water (Byrd et al., 2003). This treatment did not affect consumption, yet significantly reduced the incidence of *Salmonella* in the crop and ceca of broilers (Byrd et al., 2003). Feed producers have used a variety of treatments to reduce pathogens in feed, including chemicals, heat and irradiation (Doyle and Erickson, 2006). Hinton and colleagues (2000) findings indicate that providing a glucose-based cocktail to broilers that are denied access to feed will maintain the crop's natural ability to inhibit the growth of *S. typhimurium* and other *Enterobacteriaceae* during feed withdrawal and therefore, play a role in reducing the number of food-borne illnesses associated with poultry by reducing the number of *Enterobacteriaceae* carried in the crop.

Another area of possible contamination occurs during transport. A study conducted by Rigby and colleagues (1980) failed to isolate salmonellae during the growing period which indicates that residual contamination was not present in the barns, probably due to the fact that it was fumigated between flocks. So it was assumed the entire flock remained, as far as could be determined, free of salmonellae through the growing period (Rigby et al., 1980). This allowed the researchers to identify transportation crates to be an important source of salmonellae contamination. The results of this study clearly indicate that *Salmonella* contamination of crates can lead to

Salmonella contamination of birds entering the plant, of the plant itself and of processed carcasses (Rigby et al., 1980). It has been suggested that positive flocks be processed at the end of the shift to reduce contamination. If transportation crates are contaminated the "clean" flocks may no longer be "clean" after the journey to the plant. This adds another hurdle to reduce paratyphoid *Salmonella* levels before processing.

A major area of research is and has been in poultry litter amendments. These amendments are intended to do many things such as reduce ammonia levels in the house, lower the water activity and reduce pathogens. Reducing pathogen populations in the litter is also very important when the litter is removed and used as fertilizer (Line, 2002). Potential contamination of irrigation water through runoff and subsequent contamination of minimally processed foods such as fresh fruits and vegetables is an increasing food safety concern (Line, 2002). This has recently been witnessed with foods such as spinach, tomatoes, cantaloupes and jalapeños which were recalled and taken off the grocery stores shelves due to bacterial contamination.

Another alternative that has been researched to reduce paratyphoid *Salmonella* levels in broiler houses deals with maintaining proper litter conditions. Observations suggest that limiting the water activity (Aw) in the litter base of the broiler house may create a less favorable environment for the multiplication of paratyphoid *Salmonella* and thus a more hygienic environment for boiler production (Carr et al., 1995). From previous research, Carr and colleagues (1995) were able to determine the parameter that had the most significant correlation to on-farm drag-swab paratyphoid *Salmonella* status was Aw, a measure of free molecular water or equilibrium relative humidity. Nine of

the farms had Aw values ≥ 0.899 with drag swab result positive at an incidence ranging from 7-100%, thus indicating that litter in this range has high probability of being positive for paratyphoid *Salmonella* (Carr et al., 1995). Hayes and colleagues (2000) propose that by maintaining poultry litter Aw below 0.84, with moisture content (MC) between 20.0 and 25.0% via drying of the litter by ventilation or other means, paratyphoid *Salmonella* populations can be controlled within commercial poultry houses. According to this research, broiler house designs should be constructed in a manner that will serve to reduce the Aw of the litter. House design is not the only factor to help lower Aw. Broiler house management is also key. Leaks should be fixed immediately, caked and wet litter should be removed from the house and proper ventilation are all factors to help reduce the Aw of the litter.

Water activity is not the only factor scientists are researching to reduce the incidence of paratyphoid *Salmonella* on broiler farms. The pH of the litter has been shown to be a major factor in the growth and survival of pathogens. Because many of these litter amendments are acidic compounds, it is reasonable to suspect that proper application could significantly lower pH and water activity of poultry litter, conditions that directly affect the survivability of microorganisms present in the litter (Line, 2002). Acidic poultry litter amendments reduce volatile ammonia by reducing pH, which shifts the NH_4/NH_3 equilibrium toward NH_4 (Line, 2002). High ammonia levels evolved from litter inside broiler houses promote stressful conditions for broilers, which may lead to respiratory diseases, decrease immunity, increased susceptibility to avian and human pathogen colonization, and a decrease in overall bird health (Line and Bailey, 2006).

Reducing ammonia levels is not only good for the broilers but also the farm worker's health. Litter amendments are commonly applied before bird placement, thereby reducing litter pH during the first week of rearing when the birds are the most susceptible to pathogen invasion (Payne et al., 2007). Payne and colleagues' (2007) data suggest that by reducing litter pH to 4, paratyphoid *Salmonella* populations can be reduced below detectable limits within 20 h or less when litter is previously contaminated with high populations of paratyphoid *Salmonella* ($\sim 10^7$). Once the birds are placed, the pH of the litter over time will start to rise towards a more neutral environment due the organic material and other factors present in the house. This may lead to the reapplication of litter treatments throughout the growout of the birds. Ivanov (2001) agrees through research experiments that lowering the pH (below 5.0) will create unfavorable conditions for the growth of ammonifying bacteria, *E. coli* and salmonellae.

Fanelli and colleagues (1969) research trails on salmonellae persistence in poultry litter suggest that a cycling of salmonellae between litter and intestine plays a significant role in maintaining intestinal infection. The results gathered indicate that frequent changing of litter or use of built-up litter could materially reduce the infection rate of flocks on litter (Fanelli et al., 1969). Corrier and colleagues (1992) results indicated that the used litter contained intestinal flora that effectively controlled paratyphoid *Salmonella* cecal colonization. Furthermore, the flora remained viable and protective in the litter after an extended period of storage and disuse (Corrier et al., 1992).

Overall farm management is a major factor to help prevent contamination of farms. Bailey and colleagues (2001) data showed high recovery rates from boot swabs (12%) and the outside dirt (6.1%) near the entrance doors to the houses show how easily movement and cross-contamination can occur and point out the need for an effective foot-bath system. Various sanitation practices, the following, will help control contamination.

- Clean housing and equipment between flocks including disinfection of water supply system;
- Remove all manure between flocks;
- Minimize exposure of equipment, feed, and flock to wild animals (birds, rodents, etc.);
- Restrict and minimize traffic onto the farm, into the houses, and between flocks;
- Utilize vehicle and personnel sanitation stations (change footwear; boot dip disinfection); and
- Clean and disinfect transport crates and vehicles after every use (Doyle and Erickson, 2006).

An additional control mechanism used to reduce/eliminate paratyphoid *Salmonella* from broilers is concept known as competitive exclusion or the Nurmi concept. Once a bird is infected with a pathogen, the bird can potentially become an asymptomatic carrier and shed the pathogen throughout its lifetime. Competitive exclusion is a phenomenon whereby feces from salmonellae-free birds, or a mixed fecal

culture of bacteria, are given to young chicks so that they will colonize the same intestinal sites that salmonellae employ and, thus, exclude the subsequent attachment of salmonellae or other enteropathogens (Jay et al., 2005). The enteropathogen-free biota may be administered orally to newly hatched chicks through drinking water or by spray inoculation in the hatchery (Jay et al., 2005). Protection is established within a few hours and generally persists throughout the life of the fowl or as long as the biota remains undisturbed (Jay et al., 2005). Essentially, the birds are receiving a "probiotic" that will help serve as protective microflora against pathogens or other undesirable microorganisms. Newly hatched chicks do not have any bacteria in their intestinal tract. If paratyphoid *Salmonella* is present, it will mostly likely colonize in the intestine since few numbers are needed. On the other hand, adult birds have extensive microorganisms in their intestinal tract and are considered to be relatively resistant to paratyphoid *Salmonella* colonization. The microflora from the adult bird will colonize the chick's intestine much faster and this allows the chick to have a greater resistance to paratyphoid *Salmonella*.

A variety of promising intervention practices have been developed that include genetic selection of animals resistant to pathogen colonization, effective sanitation practices for the farm and transportation environments, feed or water amendments to reduce pathogen contamination, and animal treatments to reduce pathogen colonization (Doyle and Erickson, 2006). For these interventions/strategies to be useful they must fulfill 3 criteria: 1) be efficacious, 2) be practical and 3) be safe and not interfere with animal's growth and development (Doyle and Erickson, 2006). A cure all product has

not been introduced to the market, so until then a combination of intervention strategies with proper management will most likely need to be implemented to help control pathogens.

Conclusion

Each year an estimated 76 million Americans become ill from consuming foods contaminated with pathogenic microbes and their toxins (Mead et al., 1999). Contamination of poultry products by paratyphoid *Salmonella* has increasingly contributed to significant human food-borne illness, with an estimated annual incidence of 1 million to 4 million cases in the United States (Tauxe, 1996). Poultry product contamination poses both human and financial risks (Hayes, 2000). Loss of consumer confidence as a result of outbreaks has also been of concern to the poultry industry (Hayes, 2000).

To help combat this ever growing concern, the poultry industry and academia is researching alternatives to reduce paratyphoid *Salmonella* contamination at the farm level. A variety of promising intervention practices have been developed that include genetic selection of animals resistant to pathogen colonization, effective sanitation practices for the farm and transportation environments, feed or water amendments to reduce pathogen contamination, and animal treatments to reduce pathogen colonization (Doyle and Erickson, 2006). Litter amendments have also been invented and tested to lower the pH and Aw of the litter so it does not support paratyphoid *Salmonella* growth or survival. Demonstration of significant pathogen-reducing capabilities for products

now on the market and currently in use for other purposes would help to rapidly fill a need for additional food safety intervention methods suitable for application during poultry production (Line, 2002). The best management strategy at the current time is a combination of intervention strategies with proper management to control on farm pathogens.

The research described further in this thesis investigates the efficacy of acidic calcium sulfate and clay or Diatomaceous Earth litter formulations against *Salmonella* in broilers.

CHAPTER III

GROWTH CHARACTERISTICS OF BROILERS ASSOCIATED WITH LITTER AMENDMENTS

Introduction

Food safety is rapidly becoming a major issue for the poultry industry as well as the food production industry as a whole. Each year an estimated 76 million Americans become ill from consuming foods contaminated with pathogenic microbes and their toxins (Mead et al., 1999). Poultry product contamination poses both human and financial risks (Hayes, 2000). Consumer confidence is dropping in products and this increases the need for interventions to reduce microbial contamination. The poultry industry is mainly concerned with *Salmonella* and *Campylobacter* since these pathogens are commonly found on raw poultry products. Paratyphoid *Salmonella* is a pathogen of main concern due to the fact that isolations from humans in the U.S. have risen steadily since 1955 and a fourfold increase had accrued by the 1990s (Center for Disease Control, 1992).

Many chemical interventions and programs have been developed to reduce bacterial numbers in processing plants. In 1996, a baseline study assessing the incidence of *Salmonella* on broilers following immersion chilling found carcass incidence to be approximately 20% (FSIS, 1998). Since then, a program called HACCP has been mandated and implemented in all poultry processing facilities. Largely due to the implementation of HACCP programs, the overall incidence of paratyphoid *Salmonella*

on broiler carcasses has since dropped to 6.1% in 2001 (Bailey et al., 2001). This microbial reduction in processing facilities aids in reducing the overall microbial load but reducing incoming microbes is the new focus of the industry.

To successfully meet federal and processing plant pathogen-control standards, recent interest has centered on the implementation of on-farm pathogen reduction programs to reduce contamination loads in and on birds entering the processing plant (Payne et al., 2007). This would allow for reduced numbers bacteria to enter the processing plant and therefore allow fewer bacteria to leave the plant on the final product. Litter amendments are one solution to the on farm contamination of microorganisms. Many formulations have been developed and tested. Because many of these products are acidic compounds, it is reasonable to suspect that proper application could significantly lower pH and water activity of poultry litter, conditions that directly affect the survivability of microorganisms present in the litter (Line, 2002). These products must not interfere with normal growth characteristics and must not present any additional risks to the broilers or farm employees. Reducing pathogen populations in the litter could, in turn, reduce horizontal transmission of pathogens among the flock and result in a lower frequency of intestinal colonization and fewer bacteria on the broiler carcasses entering the processing facility (Line, 2002).

The purpose of this study was to determine if the introduction of acidic calcium sulfate with either Diatomaceous Earth (DE) or hydrated sodium calcium aluminiosilicate (Clay) would interfere with normal growth characteristics of broilers during growout.

Materials and Methods

All of the birds in this study were given free access to feed and water. The feed was weighed out in grams to allow for feed conversion calculations and the end of the trial. The poultry barn which was used for the experiment was divided into 9 pens, each equipped with nipple waterers and appropriate feeders. A starter diet was fed the first 3 weeks of the study and then the broilers were switched to a grower diet for the remainder of the study.

Each of the litter amendments were applied to randomly selected pens throughout the barn. Three pens per litter amendments were used for replication purposes. Experimental group 1 consisted of the diatomaceous earth and acid mixture, experimental group 2 was the clay and acid mixture and experimental group 3 was the control for the experiment. Each litter amendment was applied by sprinkling the formulation on the litter and then raked throughout the pen to maximize pen coverage.

After litter application, 135 one (1) day old chicks were wingbanded, weighed in grams and 15 chicks were randomly placed in each pen. Every week weights were recorded for each bird to determine growth rates. The experiment started with day old chicks and lasted 5 weeks and 3 days.

Statistics

The statistics for body weight comparison were performed using the GLM and PDIFF procedure in SASv9.1. The statistics for feed conversion were performed using Duncan's multiple range test in SASv9.1.

Results

The initial body weights of the day old chicks showed no interaction between the experimental groups and the replication (table 1). There were however significant differences between the experimental groups. Experimental group 2 (clay+acid) was significantly different from experimental group 3 (control).

Week 1 body weights showed a significant difference between experimental group 2 (clay+acid) and experimental groups 1 (DE+acid) and 3 (control) (table 1). There was no interaction between the experimental group and replication. Experimental group 2 (clay+acid) chicks had the highest body weight compared to the other experimental group.

Body weights from week 2 statically were the same as week 1 (table 1). That is experimental group 2 (clay+acid) was significantly different from experimental groups 1 (DE+acid) and 3 (control). Again, there was no statistical interaction between the replication and experimental group. The clay+acid, experimental group 2, birds showed a higher body weight than the other two experimental groups.

Week 3 (table 1) and week 4 (table 1) body weights followed the same pattern as the previous weeks with experimental group 2 (clay+acid) being statistically significantly different from experimental groups 1 (DE+acid) and 3 (control). There was no interaction between the experimental group and replication in these weeks. Again, experimental group 2 (clay+acid) broilers had a higher body weight than the other experimental groups.

The body weights for week 5 showed no interaction between the experimental group and replication (table 1). Although, experimental group 2 (clay+acid) was significantly different from experimental group 3 (control).

Experimental groups 1 (DE+acid) and 2 (clay+acid) were significantly different from experimental group 3 (control) in week 6 body weights (table 1). There was also an interaction present between the experimental group and replication. This interaction is due to water line problems at the poultry center where the project was being conducted. Water mains were turned on and off throughout a period of 2 days. Experimental group 2 (clay+acid) broilers exhibited the highest body weight at the end of the trial.

Another factor that was analyzed was the feed conversion for each individual experimental group (table 2). Experimental group 1 (DE+acid) had the lowest feed conversion of 1.773. Experimental group 2 (clay+acid) had the second lowest feed conversion while the control, experimental group 3, had the highest feed conversion value in the trial.

TABLE 1. Body weight gain in grams from initial placement through 6 weeks

Experimental Group	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*	*Week 6*
DE + Acid	39.1[ab]	103.1[b]	287.5[b]	625 [b]	1131[b]	1726.7[ab]	1793.6[a]
Clay + Acid	39.8[a]	113.3[a]	323.9[a]	680.2[a]	1222[a]	1807.5[a]	1829.8[a]
Control	38.6[b]	99[b]	291 [b]	628.1[b]	1104.7[b]	1703.8[b]	1673.8[b]
Standard Error	0.233	1.568	4.747	9.541	15.180	20.936	23.816

[a-b] Means within a column and experimental group with different superscripts differ significantly ($P < 0.05$)

TABLE 2. Feed conversion ratios

Experimental Group	*Feed Conversion Ratio*
DE + Acid	1.77
Clay + Acid	1.79
Control	1.86
Standard Error	0.038

Discussion

Both litter amendments, DE+acid and clay+acid, had significantly higher body weights than the control broilers at the end of the study. The data collected from this study was able to prove that litter formulations consisting of acidic calcium sulfate with either clay or Diatomaceous Earth do not have any negative effects on normal growth characteristics of broilers. The data supports that broilers grown on litter treated by either litter amendment were significantly heavier. Further research needs to be conducted on a large scale to validate these results.

CHAPTER IV

SALMONELLA P RESENCE OR CFU/G IN BROILER LITTER AND BROILER CROPS AND CECAS ASSOCIATED WITH LITTER AMENDMENTS

Introduction

Salmonella is a small, motile, non-sporeforming, gram negative rod. It is part of the *Enterobacteriaceae* family, which also includes *Escherichia* and *Shigella*. The genus *Salmonella* includes three habitat types with approximately 2400 serotypes. One habitat type infects humans only. Examples of these serotypes include *S. typhi* (Typhoid Fever) and *S. paratyphi* (Paratyphoid Fever). Another type of habitat is labeled as host-adapted. These serotypes are adapted to specific animal hosts. For example, *S. Pullorum & S. Gallinarum* are found in poultry and *S. Dublin* is found in cattle. The third habitat is known as the non host-adapted which are known as paratyphoid *Salmonella*. . These serotypes are pathogenic for humans and animals. The non-host adapted *Salmonella* typically cause gastroenteritis. This is the most common form of salmonellosis found in the United States. Symptoms of this illness include diarrhea, abdominal pain, vomiting and dehydration. The incubation of this microorganism for foodborne illness is usually between 5 and 72 hours. The duration of the illness is anywhere from 1 to 4 days.

These non-host adapted *Salmonella* serotypes are what the poultry industry is concerned about since raw poultry products are known to harbor this pathogen.

Contamination of poultry products by paratyphoid *Salmonella* has increasingly contributed to significant human foodborne illness, with an estimated annual incidence of 1 million to 4 million cases in the United States (Tauxe, 1996). The increase in foodborne illness cases, federal regulations and loss of consumer confidence is what prompted the industry to try to lower paratyphoid *Salmonella* counts on their products.

To successfully meet federal and processing plant pathogen-control standards, recent interest has centered on the implementation of on-farm pathogen reduction programs to reduce contamination loads in and on birds entering the processing plant (Payne et al., 2007). This would allow for reduced numbers of bacteria to enter the processing plant and therefore allow fewer bacteria to leave the plant on the final product. These on-farm pathogen reduction strategies range from better management practices to chemical interventions. Litter amendments are one strategy that has been developed and researched to try to lower the number of microorganisms found in the poultry litter. Because many of these products are acidic compounds, it is reasonable to suspect that proper application could significantly lower pH and water activity of poultry litter, conditions that directly affect the survivability of microorganisms present in the litter (Line, 2002). This allows the growers to produce "cleaner" birds, lower amounts of microorganisms, and will allow for less carcass contamination in processing facilities.

The objective of this study is the determination of two types of litter amendment formulations on *S.* Typhimurium colonization in litter, *S.* Typhimurium colonization in crop and ceca of broilers, broiler growth characteristics and litter moisture content.

Materials and Methods

All of the birds in this study were given free access to feed and water. The feed was weighed out in grams to allow for feed conversion calculations at the end of the trail. An isolation building with 4 rooms was used during the experiment. Each room was divided into 3 pens, each being equipped with nipple waterers and appropriate feeders. The study consisted of 12 pens with 3 replications of each experimental group. A starter diet was fed the first 3 weeks of the study and then the broilers were switched to a grower diet for the remainder.

The litter amendments were first applied to randomly selected pens throughout the barn except for experimental group 1 which was the absolute control. Experimental group 4 consisted of a control with *S.* Typhimurium, experimental group 5 was a Diatomaceous Earth acid mixture with *S.* Typhimurium, and experimental group 6 was a clay acid mixture with *S.* Typhimurium. Each litter amendment was first sprinkled on the litter and then raked throughout the pen to cover the entire floor space. Once each litter amendment was applied, 150ml of *S.* Typhimurium was applied to each pen except for the absolute control at a concentration of 3.8×10^8 cfu/ml using a spray bottle. After this, each day old chick was wingbanded, weighed in grams and 15 chicks were randomly placed in each pen. Every week body weights were recorded for each bird. The experiment started with day old chicks and lasted 5 weeks and 3 days.

Litter samples were collected every weigh day (once a week). Samples were randomly taken from various areas in each pen and placed into a Ziploc-type bag. The bag was then shaken to ensure a representative sample of the whole pen. Once the initial

samples were taken, 25 grams of each litter sample was weighed out a placed into Whirl-Pak® bags. 50ml of Butterfield's Buffer solution was then added to the bags. Then 0.1ml from the Whirl-Pak® bag was spread plated directly onto a BGS plate (10^0 dilution). After this, 1ml was taken from the Whirl-Pak® bag and dispensed into a 9ml dilution tube of Butterfield's Buffer solution (10^{-1} dilution). Another 1ml was taken from the 10^{-1} dilution tube and placed in a 9ml dilution tube of Butterfield's Buffer solution (10^{-2} dilution). Once the dilutions were completed, 0.1ml of the 10^{-1} and 10^{-2} dilutions were spread plated onto separate BGS plates. Then an additional 1ml from the Whirl-Pak® bag sample was dispersed into a 9ml tube of Tetrathionate Broth, which was used for enrichment purposes. This procedure was carried out for all 12 pens each week. Both the BGS plates and the Tetrathionate Broth were incubated for 18-24 hours at 35-37^{o}C. Each step was duplicated to ensure accuracy. After incubation, the BGS plates were read and the Tetrathionate Broth sample was streak plated onto BGS plates. The enrichment plates were again incubated for 18-24 hours at 35-37^{o}C. Once the appropriate incubation time passed, the Tetrathionate plates were read. If the media turned pink, *Salmonella* was present.

At 3 weeks and 5 ½ weeks post placement, 6 birds from each pen were euthanized using CO_2 and tested for the presence or cfu/g of crop/ceca of *S.* Typhimurium. The wingband numbers were recorded and the bird's crop and cecal tonsils were aseptically removed. The crop and ceca were placed into separate Whirl-Pak® bags and diluted with a 1:2 ratio by weight by adding Butterfield's Buffer solution to each bag. Once the Butterfield's Buffer solution was added, the bags were then

stomached for 30 seconds. Then 0.1ml from the sample bag was dispensed into a 9ml dilution tube with Butterfield's Buffer solution (10^{-2} dilution). Then another 1ml sample was taken from each sample bag and dispensed into a 9ml Tetrathionate Broth tube (enrichment). Next, 1ml from each of the 10^{-2} Butterfield's Buffer solution dilutions was dispensed into the appropriate 9ml dilution tube of Butterfield's Buffer solution (10^{-3} dilution). After that, 1ml from each of the 10^{-3} Butterfield's Buffer solution dilution tubes was pipetted into the appropriate 9ml dilution tube of Butterfield's Buffer solution (10^{-4} dilution). Then 0.1ml from each of the 10^{-2}, 10^{-3}, and 10^{-4} dilutions was spread plated onto appropriate BGS plates. The BGS plates and the Tetrathionate Broth tubes were then incubated at 35-37^{o}C for 18-24 hours. After the allotted incubation time, the BGS plates were read and the Tetrathionate Broth sample was streaked onto BGS plates. The Tetrathionate plates were then incubated again for 18-24 hours at 35-37^{o}C. Once this time passed, the enrichment plates were read. If the Tetrathionate plates were positive for *Salmonella,* the media would turn pink in color.

After the project concluded, total pen weights were analyzed and replications for each experimental group could then be grouped together. Feed conversion ratios were also calculated for the growout. *S.* Typhimurium data included each weeks litter samples and the crop and ceca samples from 6 birds at 3 and 5 ½ weeks.

If there was no growth on the direct BGS plates, enrichment plates were viewed to determine qualitative results. Positive plates from enrichment were assigned a $\log_{10}$ value of 1.5 (Corrier et al., 1993).

Statistics

All statistics were run using SAS v9.1. Statistical tests used for litter counts included PROC GLM, MEANS statements and Duncan's Multiple Range Test. The statistical tests used for crop and ceca counts included PROC GLM, LS MEANS and PDIFF.

Results

The body weight comparison for all birds in the study from the time period of initial placement to week 3 showed significant differences in week 1 and week 3 (table 3). In week 1, the positive control was significantly different from the clay + *Salmonella* treatment. Week 3 body weights showed that the negative control was significantly lighter than the other treatment types in the study.

The body weight comparison for the birds that were not sacrificed at week 3 can be seen in table 4. The time period was from the initial placement to week 6. There were no significant differences in week 0, 1, 2 or 5. Significant differences were found in week 3, 4 and 6. The negative control was significantly lighter than the other experimental groups. The DE + *Salmonella* experimental group birds showed an overall increased body size starting at week 4 and this trend was carried out throughout the remainder of the study.

There was no significant difference found in the *S.* Typhimurium cfu/g in broiler litter throughout the entire study (week 1-6) (table 5). *S.* Typhimurium cfu/g in litter were relatively low considering the amount that was first applied to the litter to begin the

trial. The *S.* Typhimurium cfu/g in litter coincides with normal growth curves for the pathogen.

The cfu/g of *S.* Typhimurium in the crop at week 3 showed no significant difference between the experimental groups (table 6). There was however, a significant difference in the ceca at this time. The positive control had the highest counts, which should be expected since these pens were inoculated with the pathogen. At this time, DE had lower counts in the ceca compared to the clay experimental group.

The cfu/g of *S.* Typhimurium in the crop at week 6 again showed no significant differences (table 6). There was a significant difference in the ceca between the DE experimental group and the rest of the experimental groups in the study. Week 6 ceca data showed the opposite effect from week 3. This time, the DE experimental group had significantly higher counts compared to the clay experimental group.

There was no significant differences found in litter moisture comparison or feed conversion (table 7). When looking at litter moisture, the DE experimental group had the highest moisture content compared to the other t experimental groups.

TABLE 3. Body weight comparisons in grams for all birds from initial placement to week 3

Experimental Group	*Week 0*	*Week 1*	*Week 2*	*Week 3*
Negative Control	49.08[a]	130.94[b]	329.57[a]	552.38[b]
Positive Control	48.08[a]	132.86[ab]	328.00[a]	627.01[a]
DE + *Salmonella*	48.85[a]	136.05[ab]	341.78[a]	617.73[a]
Clay + *Salmonella*	49.24[a]	141.92[a]	350.62[a]	624.20[a]
Standard Error	0.291	1.880	4.542	10.150

[a-b] Means within a column and experimental group with different superscripts differ significantly ($P < 0.05$)

TABLE 4. Body weight comparisons in grams for birds not sacrificed at week 3 for weeks 0-6

Experimental Group	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*	*Week 6*
Negative Control	49.56[a]	132.33[a]	329.54[a]	551.17[b]	1087.22[b]	1754.88[a]	2044.33[ab]
Positive Control	47.22[a]	132.82[a]	327.53[a]	625.98[a]	1123.71[ab]	1709.80[a]	1975.58[b]
DE + *Salmonella*	48.96[a]	138.89[a]	343.58[a]	640.91[a]	1173.60[a]	1821.14[a]	2146.30[a]
Clay + *Salmonella*	48.95[a]	140.16[a]	348.79[a]	631.09[a]	1154.55[ab]	1760.90[a]	2074.67[ab]
Standard Error	0.428	2.479	5.526	11.558	18.090	26.987	30.248

[a-b] Means within a column and experimental group with different superscripts differ significantly ($P < 0.05$)

TABLE 5. *Salmonella* litter recovery weeks 0-6 as average $\log_{10}$ cfu/g of litter

Experimental Group	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*	*Week 6*
Negative Control	1	0.5	0	1.777	2.513	0.487
Positive Control	0.853	0.9	0.97	1.173	3.06	0.323
DE + *Salmonella*	0.777	1.607	1.137	1.063	3.066	1.123
Clay + *Salmonella*	1.78	0.51	0.86	1.123	2.747	0.62
Standard Error	0.274	0.218	0.233	0.256	0.149	0.145

TABLE 6. *Salmonella* crop and ceca recovery as average $\log_{10}$ cfu/g of crop/ceca at weeks 3 and 6

Experimental Group	*Week 3 Crop*	*Week 6 Crop*	*Week 3 Ceca*	*Week 6 Ceca*
Negative Control	0^{a}	0^{a}	0^{b}	0.08^{b}
Positive Control	0.08^{a}	0.08^{a}	0.75^{a}	0.08^{b}
DE + *Salmonella*	0^{a}	0.08^{a}	0.25^{bc}	0.4^{a}
Clay + *Salmonella*	0^{a}	0.08^{a}	0.51^{ac}	0^{b}
Standard Error	0.028	0.047	0.681	0.270

[a-c] Means within a column and experimental group with different superscripts differ significantly ($P < 0.05$)

TABLE 7. Litter moisture comparison and feed conversion ratios

Experimental Group	*Percent (%) Moisture*	*Feed Conversion Ratios*
Negative Control	18.171	1.8018
Positive Control	17.283	1.7118
DE + *Salmonella*	22.663	1.7174
Clay + *Salmonella*	16.245	1.7017
Standard Error	2.591	0.034

Discussion

The efficacy of the litter amendments varied throughout the research. Further research is needed on the litter amendments to make any further conclusions. The DE experimental group had lower counts in the ceca at 3 weeks but higher counts in the ceca at 6 weeks. A possible reason for the low *S.* Typhimurium counts could be due to the low moisture of the litter and optimal growing conditions in isolation rooms.

CHAPTER V

SALMONELLA P RESENCE OR CFU/G IN BROILER LITTER ASSOCIATED WITH FEEDING SODIUM BISULFATE

Introduction

Foodborne pathogens, such as paratyphoid *Salmonella,* continue to be a major concern for the food industry. Contamination of poultry products by paratyphoid *Salmonella* has increasingly contributed to significant human foodborne illness, with an estimated annual incidence of 1 million to 4 million cases in the United States (Tauxe, 1996). Since paratyphoid *Salmonella* has been linked to the consumption of contaminated poultry products (Tauxe, 1991; Bryan and Doyle, 1995; Hoszowski et al., 1996; Byrd et al., 1997; Cox et al., 2000) it has developed into a major concern for the industry as a whole.

The integration of the HACCP (Hazardous Analysis Critical Control Points) system into poultry processing facilities has drastically reduced the number of foodborne pathogens leaving the plant on poultry products. This mandatory program has helped reduce pathogen numbers in processing facilities, but the next step is to reduce pathogen numbers on the growout farms. The idea is to reduce the amount of microorganisms, specifically pathogens, entering the plant. This would allow for less contamination entering the processing plant and potentially reduce the amount of microorganisms leaving the plant on the final product which will enter commerce.

On-farm pathogen reduction strategies have been developed to try to lower the amount of microorganisms found on growout farms. These on-farm pathogen reduction strategies range from better management practices to chemical interventions. Litter amendments are one strategy that has been developed and researched to try to lower the number of microorganisms found in the poultry litter. Another method to reduce microorganism numbers includes the addition of specifically designed feed ingredients.

The objective of this research is to determine the efficacy of feeding differing amounts of feed grade Sodium Bisulfate on *Salmonella* levels, aerobic plate counts and pH in poultry litter.

Materials and Methods

The experiment consisted of 3 experimental groups. Experimental group A contained 0.25% Sodium Bisulfate added to a commercial poultry diet, experimental group B contained 0.75% Sodium Bisulfate added to a commercial poultry diet and experimental group C was a standard commercial poultry diet for the control.

All of the birds in this study were given free access to feed and water. A poultry research barn was used at the Texas A&M University Poultry Science Center. The barn was divided into 18 pens, each being equipped with nipple waterers and appropriate feeders. The study consisted of 18 pens with 4 replications of each experimental group. A starter diet was fed the first 3 weeks of the study and then the broilers were switched to a grower diet for 2 weeks and then a finisher diet for the remainder of the study.

Litter samples were collected once a week. Samples were randomly taken from various areas in each pen and placed into a Ziploc-type bag. The bag was then shaken to ensure a representative sample of the whole pen. Once the initial samples were taken, 25 grams of each litter sample was weighed out a placed into Whirl-Pak® bags. 50ml of Butterfield's Buffer solution was then added to the bags. Then 0.1ml from the Whirl-Pak® bag was spread plated directly onto a XLT4 plate (10^0 dilution). After this, 1ml was taken from the Whirl-Pak® bag and dispensed into a 9ml dilution tube of Butterfield's Buffer solution (10^{-1} dilution). Another 1ml was taken from the 10^{-1} dilution tube and placed in a 9ml dilution tube of Butterfield's Buffer solution (10^{-2} dilution). Once the dilutions were completed, 0.1ml of the 10^{-1} and 10^{-2} dilutions were spread plated onto separate XLT4 plates. Then an additional 1ml from the Whirl-Pak® bag sample was dispersed into a 9ml tube of RV, which was used for enrichment purposes. This procedure was carried out for all 18 pens each week. The XLT4 plates were incubated for 18-24 hours at 35-37^{o}C. The RV tubes were incubated for 18-24 hours at 42^{o}C. After incubation, the XLT4 plates were read and the RV samples were streak plated onto XLT4 plates. The enrichment plates were again incubated for 18-24 hours at 35-37^{o}C. Once the appropriate incubation time passed, the RV plates were read.

The same dilution process was also used to determine the aerobic plate count using PCA. These plates were incubated for 18-24 hours at 35-37^{o}C. Then the colonies were counted and recorded. Aerobic plate counts were also performed on feed samples.

Another factor that was analyzed each week was the pH of the litter. In a Whirl-Pak® bag, 3 grams of litter was added to 50 ml of deionized water (Coufal et al., 2006).

The pH probe was then placed in to bag and the resulting pH was then recorded. This was also performed on feed samples.

If there was no growth on the direct BGS plates, enrichment plates were viewed to determine qualitative results. Positive plates from enrichment were assigned a $\log_{10}$ value of 1.5 (Corrier et al., 1993).

Statistics

The first trial statistics were run using SAS v9.1. Statistical tests used included PROC GLM and Duncan's Multiple Range Test. The second trial statistics were run using SPSS. The statistical tests used for litter counts and pH included Analysis of Variance and Duncan's Multiple Range Test.

Results

The aerobic plate counts for trial 1 (table 8) showed no significant differences for week 0-7. There were very few countable colonies for both experimental groups and control early in the experiment.

Salmonella cfu/g in litter for trial 1 (table 9) had significant differences in weeks 4 and 5. The 0.25% Sodium Bisulfate and control experimental groups were significant different from the 0.75% Sodium Bisulfate experimental group in week 4 of the trial. Week 5 showed a significant difference between the 0.25% Sodium Bisulfate experimental group and the control.

The pH of the litter for trial 1 (table 10) showed significant differences in weeks 4-6. Week 4's data showed a significant difference between the 0.25% Sodium Bisulfate experimental group and the 0.75% Sodium Bisulfate and control experimental groups. Week 5 had a significant difference between the 0.75% Sodium Bisulfate experimental group and the control and week 6 showed a significant difference between the 0.25% Sodium Bisulfate experimental group and the 0.75% Sodium Bisulfate experimental group.

The percent moisture for trial 1(table 11) increased over time as expected. The only significant difference exhibited in this parameter was in week 7. The significant difference was seen between the 0.25% Sodium Bisulfate experimental group and the 0.75% Sodium Bisulfate experimental group.

The aerobic plate counts for trial 2 (table 8) had no significant differences for weeks 0-7.

Salmonella cfu/g in litter for trial 2 (table 9) showed significant differences in week 4's data. The 0.25% Sodium Bisulfate and control experimental groups were significant different from the 0.75% Sodium Bisulfate experimental group.

The pH data from trial 2 (f table 10) had significant differences in weeks 4 and 5. In week 4, the 0.25% Sodium Bisulfate and 0.75% Sodium Bisulfate experimental groups were significantly different from the control experimental group. Week 5's data showed a significant difference between the 0.75% Sodium Bisulfate experimental group and the control.

TABLE 8. APC recovery as average $\log_{10}$ cfu/g of litter trials 1 and 2

Experimental Group	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*	*Week 6*	*Week 7*
TRIAL 1								
0.25% Sodium bisulfate	3.43	3.80	7.67	8.66	9.40	10.86	11.02	9.75
0.75% Sodium bisulfate	3.67	3.79	7.90	9.23	9.65	8.41	10.91	10.81
Control	3.58	3.78	8.34	8.64	9.66	10.65	11.06	10.54
Standard Error	2.61	2.61	8.03	8.44	8.72	10.36	10.46	10.25
TRIAL 2								
0.25% Sodium bisulfate	6.01	6.50	7.01	7.24	8.07	8.57	7.24	6.97
0.75% Sodium bisulfate	6.11	6.25	7.08	7.39	7.80	7.86	6.92	7.26
Control	6.07	6.57	7.20	6.84	8.10	7.71	7.48	6.69
Standard Error	0.112	0.273	0.353	0.286	0.424	0.409	0.365	0.333

TABLE 9. *Salmonella* recovery as average $\log_{10}$ cfu/g of litter trials 1 and 2

Experimental Group	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*	*Week 6*	*Week 7*
TRIAL 1								
0.25% Sodium bisulfate	1.22	2.77	0.91	2.19	0.93[b]	1.69	2.08[b]	1.92
0.75% Sodium bisulfate	0	1.76	0	2.08	1.50[a]	2.27	2.49[ab]	2.65
Control	0.52	0.95	2.08	1.24	1.10[b]	1.64	3.01[a]	2.26
Standard Error	0.74	2.18	1.57	1.64	0.60	1.68	2.22	2.17
TRIAL 2								
0.25% Sodium bisulfate	0.80	1.27	2.23	2.50	2.50[b]	2.79	2.48	1.63
0.75% Sodium bisulfate	1.20	1.38	2.97	2.49	3.10[a]	2.99	2.79	1.82
Control	0.87	0.97	2.21	2.67	2.29[b]	2.25	2.28	2.25
Standard Error	0.28	0.18	0.40	0.19	0.22	0.24	0.28	0.36

[a-b] Means within a column and experimental group with different superscripts differ significantly ($P < 0.05$)

TABLE 10. pH of litter trials 1 and 2

Experimental Group	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*	*Week 6*	*Week 7*
TRIAL 1								
0.25% Sodium bisulfate	8.10	7.38	7.35	8.27	7.95[b]	8.80[ab]	8.78[a]	8.90
0.75% Sodium bisulfate	7.57	7.62	7.02	8.04	8.48[a]	8.71[b]	8.49[b]	8.77
Control	7.89	7.75	7.74	8.22	8.42[a]	8.85[a]	8.65[ab]	8.94
Standard Error	0.126	0.110	0.153	0.089	0.098	0.026	0.043	0.040
TRIAL 2								
0.25% Sodium bisulfate	7.99	7.83	7.15	7.96	8.70[a]	8.88[ab]	8.84	8.86
0.75% Sodium bisulfate	7.92	7.87	7.05	8.45	8.72[a]	9.01[a]	8.88	8.99
Control	7.90	7.81	7.57	8.69	8.16[b]	8.56[b]	8.55	8.88
Standard Error	0.057	0.144	0.231	0.279	0.158	0.132	0.144	0.143

[a-b] Means within a column and experimental group with different superscripts differ significantly ($P < 0.05$)

TABLE 11. Litter moisture comparison for trial 1

Experimental Group	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*	*Week 6*	*Week 7*
0.25% Sodium bisulfate	6.54	12.14	18.43	27.86	23.09	30.78	33.34	26.70[b]
0.75% Sodium bisulfate	9.04	11.93	20.26	28.78	25.93	31.86	35.07	36.55[a]
Control	9.42	11.41	19.35	26.53	24.72	33.17	32.67	29.90[ab]
Standard Error	1.056	0.474	0.693	0.929	0.788	1.140	0.681	1.757

[a-b] Means within a column and experimental group with different superscripts differ significantly ($P < 0.05$)

Discussion

The aerobic plate counts, *Salmonella* cfu/g in litter, pH and moisture values varied throughout the trials for the experimental groups that were studied. The 0.75% Sodium Bisulfate experimental group seemed to have higher percent moisture compared to the control and 0.25% Sodium Bisulfate experimental group in trial 1. In trial 2, the 0.75% Sodium Bisulfate experimental group had higher cfu/g later in the study but ended up having the lowest cfu/g at the end of the trial (week 7). This could be beneficial since the *Salmonella* levels could possibly be lower on these birds traveling to the processing facility. The litter pH for the 0.75% Sodium Bisulfate experimental group seemed to be higher for most of trial 2.

CHAPTER VI

CONCLUSION

Food safety is never guaranteed and is sometimes taken for granted in the United States. Recently, many food recalls have prompted food safety awareness. Paratyphoid *Salmonella* continues to be a foodborne pathogen of concern and has been linked to the consumption of contaminated poultry products (Tauxe, 1991; Bryan and Doyle, 1995; Hoszowski et al., 1996; Byrd et al., 1997; Cox et al., 2000).

Poultry processing facilities have done well with reducing pathogen levels with the implementation of HACCP and the use of other interventions including chemical means. The next step to reducing the overall pathogen load leaving the processing plant is to back track to the growout farms. If microbial loads are reduced at the farm level, there will be fewer microbes entering the plant and therefore less leaving the plant on the final product.

This research focused on two types of on farm interventions to reduce *Salmonella* levels in broiler litter. The first experiment used two differing litter amendments while the other experiment consisted of feeding sodium bisulfate. Both experiments showed promise but further research is required for each study to make any further conclusions. These interventions also need to be tested on a commercial scale as well.

REFERENCES

Bailey, J. S. 1988. Integrated colonization control of *Salmonella* in poultry. Poult Sci. 67:928-932.

Bailey, J. S., N. J. Stern, P. Fedorka-Cray, S. E. Craven, N. A. Cox, D. E. Cosby, S. Ladely, and M. T. Musgrove. 2001. Sources and movement of *Salmonella* through integrated poultry operations: a multistate epidemiological investigation. J Food Prot. 64:1690-1697.

Brown, D. D., J. G. Ross, and A. F. G. Smith. 1976. Experimental infection of poultry with *Salmonella infantis*. Res Vet Sci. 20:237-243.

Bryan, F. L. and M. P. Doyle. 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J Food Prot. 58:326-344.

Byrd, J. A., R. C. Anderson, T. R. Callaway, R. W. Moore, K. D. Knape, L. F. Kubena, R. L. Ziprin, and D. J. Nisbet. 2003. Effect of experimental chlorate product administration in the drinking water on *Salmonella* Typhimurium contamination of broilers. Poult Sci. 82:1403-1406.

Byrd, J. A., D. E. Corrier, J. R. EdLoach, and D. J. Nisbet. 1997. Comparison of drag-swab environmental protocols for the isolation of *Salmonella* in poultry houses. Avian Dis. 41:709-713.

Carr, L. E., E. T. Mallinson, C. R. Tate, R. G. Miller, E. Russek-Cohen, L. E. Stewart, O. O. Opara, and S. W. Joseph. 1995. Prevalence of *Salmonella* in broiler flocks: Effect of litter water activity, house construction, and watering devices. Avian Dis. 39:39-44.

Centers for Disease Control (CDC). 2008. Multistate outbreak of *Salmonella* infection associated with peanut butter and peanut butter-containing products --- United States, 2008-2009. Morbid Mortal Weekly Rep. 58(04):85-90.

Centers for Disease Control (CDC). 2008. Multistate outbreak of *Salmonella* infection associated with frozen pot pies --- United States, 2007. Morbid Mortal Weekly Rep. 57(47):1277-1280.

Centers for Disease Control (CDC). 2008. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food --- 10 states, 2007. Morbid Mortal Weekly Rep. 57(14):366-370.

Centers for Disease Control (CDC). 2000. Outbreaks of *Salmonella* serotype Enteritidis infection associated with eating raw or undercooked shell eggs, United States, 1996-1998. Morbid Mortal Weekly Rep. 49:73-79.

Centers for Disease Control (CDC). 1992. Summary of notifiable diseases, United States, 1991. Morbid Mortal Weekly Rep. 40(53):41.

Chambers, J. R., J. R. Bisaillon, Y. Labbe, C. Poppe, and C. F. Langford. 1998. *Salmonella* prevalence in crops of Ontario and Quebec broiler chickens at slaughter. Poult Sci. 77:1497-1501.

Chambers, J. R., J. L. Spencer, and H. W. Modler. 1997. The influence of complex carbohydrates on *Salmonella typhimurium* colonization, pH, and density of broiler ceca. Poult Sci. 76:445-451.

Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, and L. H. Stanker. 1999. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawl. Poult Sci. 78:45-49.

Corrier, D. E., D. J. Nisbet, A. G. Hollister, C. M. Scanlan, B. M. Hargis, and J. R. DeLoach. 1993. Development of defined cultures of indigenous cecal bacteria to control salmonellosis in broiler chicks. Poult Sci. 72: 1164-1168.

Corrier, D. E., A. Hinton Jr., B. M. Hargis, and J. R. DeLoach. 1992. Effect of used litter from floor pens of adult broilers on *Salmonella* colonization of broiler chicks. Avian Dis. 36:897-902.

Coufal, C. D., C. Chavez, P. R. Niemeyer, and J. B. Carey. 2006. Measurement of broiler litter production rates and nutrient content using recycled litter. Poult Sci. 85: 398-403.

Cox, N. A., N. J. Stern, S. E. Craven, M. E. Berrang, and M. T. Musgrove. 2000. Prevalence of *Campylobacter* and *Salmonella* in the cecal droppings of turkeys during production. J Appl Poult Res. 9:542-545.

Doyle, M. P. and M. C. Erickson. 2006. Reducing the carriage of foodborne pathogens in livestock and poultry. Poult Sci. 85:960-973.

Fanelli, M. J., W. W. Sadler, C. E. Franti, and J. R. Brownell. 1971. Localization of Salmonellae within the intestinal tract of chickens. Avian Dis. 15:366-395.

Fanelli, M. J., W. W. Sadler, C. E. Franti, and J. R. Brownell. 1969. Preliminary studies on persistence of salmonellae in poultry litter. Avian Dis. 14:131-141.

FSIS. 1998. FSIS, FDA, CDC, State health departments collaborate on foodborne illness project.

FSIS. 1996. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) System; Final Rule. Federal Register. 9 CFR Part 304 et al 61:38868-38871.

Hayes, J. R., L. E. Carr, E. T. Mallinson, L. W. Douglass, and S. W. Joseph. 2000. Characterization of the contribution of water activity and moisture content to the population distribution of *Salmonella* spp. in commercial poultry houses. Poult Sci. 79:1557-1561.

Hinton, A., Jr., R. J. Buhr, and K. D. Ingram. 2000. Reduction of *Salmonella* in the crop of broiler chickens subjected to feed withdrawl. Poult Sci. 79:1566-1570.

Holt, P. S. and R. E. Porter, Jr. 1993. Effect of induced molting on the recurrence of a previous *Salmonella enteritidis* infection. Poult Sci. 72:2069-2078.

Hoszowski, A., A. D. E. Fraser, B. W. Brooks, and E. M. Riche. 1996. Rapid detection and enumeration of *Salmonella* in chicken carcass rinses using filtration, enrichment, and colony blot immunoassay. Int J Food Microbiol. 28:341-350.

ICMSF. 2002. Microorganisms in foods 7. New York: Kluwer Academic/Plenum Publishers.

Ivanov, I. E. 2001. Treatment of broiler litter with organic acids. Res Vet Sci. 70:169-173.

Jay, James M., Martin J. Loessner, and David A. Golden. 2005. Modern food microbiology. New York: Springer Science.

Jones, F. T., and K. E. Richardson. 2004. *Salmonella* in commercially manufactured feeds. Poult Sci. 83:384-391.

Jones, F. T., R. C. Axtell, D. V. Rives, S. E. Scheideler, F. R. Tarver, Jr., R. L. Walker, and M. J. Wineland. 1991. A survey of *Salmonella* contamination in modern broiler production. J Food Prot. 54:502-507.

Kingston, D. J. 1981. A comparison of culturing drag swabs and litter for identification of infection with *Salmonella* spp. in commercial chicken flocks. Avian Dis. 25:513-516.

Kubena, L. F., J. A. Byrd, C. R. Young, and D. E. Corrier. 2001. Effects of tannic acid on cecal volatile fatty acids and susceptibility to *Salmonella typhimurium* colonization in broiler chicks. Poult Sci. 80:1293-1298.

Kwon, H. J., K. Y. Park, H. S. Yoo, J. Y. Park, Y. H. Park, and S. J. Kim. 2000. Differentiation of *Salmonella* enterica serotype gallinarum biotype pullorum from biotype gallinarum by analysis of phase 1 flagellin C gene (fliC). J Microbiol Methods 40:33-38.

Lillard, H. S. 1989. Factors affecting the persistence of *Salmonella* during the processing of poultry. J Food Prot. 52:829-832.

Line, J. E., J. S. Bailey. 2006. Effect of on-farm litter acidification treatments on *Campylobacter* and *Salmonella* populations in commercial broiler housed in northeast Gerogia. Poult Sci. 85:1529-1534.

Line, J. E. 2002. *Campylobacter* and *Salmonella* populations associated with chickens raised on acidified litter. Poult Sci. 81:1473-1477.

Long, J. R., W. F. DeWitt, J. L. Ruet. 1980. Studies on *Salmonella* from floor litter of 60 broiler chicken houses in Nova Scotia. Can Vet J. 21:91-94.

Lowry, V. K., G. I. Tellez, D. J. Nisbet, G. Garcia, O. Urquiza, L. H. Stanker, and M. H. Kogut. 1999. Efficacy of *Salmonella* enteritidis-immune lymphokines on horizontal transmission of *S. arizonae* in turkeys and *S. gallinarum* in chickens. Int J Food Microbiol. 48:139-148.

McGarr C., W. R. Mitchell, H. C. Carlson, and N. A. Fish. 1980. An epidemiological study of salmonellae in broiler chicken production. Can J Public Health 71:47-57.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg Infect Dis. 5:607-625.

Payne, J. B., J. A. Osborne, P. K. Jenkins, and B. W. Sheldon. 2007. Modeling the growth and death kinetics of *Salmonella* in poultry litter as a function of pH and water activity. Poult Sci. 86:191-201.

Porter, R., Jr. 1998. Bacterial enteritides of poultry. Poult Sci. 77: 1159-1165.

Primm, N. D. 1998. Field experiences with the control of salmonellae introduction into turkey flocks via contaminated feeds. Proc Western Poult Dis Conf. 47:27-29.

Ramirez, G. A., L. L. Sarlin, D. J. Caldwell, C. R. Yezak, Jr., M. E. Hume, D. E. Corrier, J. R. Eloach, and B. M. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. Poult Sci. 76:654-656.

Randall, C. J. 1985. Diseases of the domestic fowl & turkey. Iowa: Iowa State University Press.

Renwick, S. A., R. J. Irwin, R. C. Clarke, W. B. McNab, C. Poppe, and S. A. McEwen. 1992. Epidemiological associations between characteristics of registered broiler chicken flocks in Canada and the *Salmonella* culture status of floor litter and drinking water. Can Vet J. 33:449-458

Rigby, C. E., J. R. Pettit, M. F. Baker, A. H. Bentley, M.O. Salomons, and H. Lior. 1980. Sources of salmonellae in an uninfected commercially-processed broiler flock. Can J Comp Med. 44:267-274.

Rigby, C. E., J. R. Pettit, M. F. Baker, A. H. Bentley, M.O. Salomons, and H. Lior. 1980. Flock infection and transport as sources of salmonellae in broiler chickens and carcasses. Can J Comp Med. 44:328-337.

Sams, A. R.. 2001. Poultry meat processing. Boca Raton: CRC Press.

Simmons, G. C., and R. V. Byrnes. 1972. The origin of salmonellas in chickens and chicken carcases. Aust Vet J. 48:186-189.

Snoeyenbos, G. H., A. S. Soerjadi, and O. M. Weinack. 1982. Gastrointestinal colonization by *Salmonella* and pathogenic *Escherichia coli* in monoxenic and holxenic chicks and poults. Avian Dis. 26:566-575.

Tauxe, R. V. 1996. An update on *Salmonella*. Health Environ 10:1-4.

USDA, Animal and Plant Health Inspection Service. 2001. National poultry improvement plan. Federal Registrer 9 CFR Part 145 72:1418.

USDA, Animal and Plant Health Inspection Service. 1994. The national poultry improvement plan and auxiliary provisions. Hyattsville, MD.

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