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The effect of dietary supplementation on Salmonella typhimurium colonization in the turkey crop

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The effect of dietary supplementation on *Salmonella typhimurium* colonization in the turkey crop

by

Sara A. Johannsen

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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2003

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Graduate College
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This is to certify that the master's thesis of

Sara A. Johannsen

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
DEDICATION

The author would like to dedicate this thesis to anyone who is not satisfied with good enough.

“All life is an experiment. The more experiments you make the better.”

Ralph Waldo Emerson
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ABSTRACT

The pre-harvest use of supplements to reduce the incidence of crop contamination in turkeys was investigated. As opposed to other studies that used a carbohydrate or lactic acid bacteria supplementation prior to slaughter in extended feed withdrawal times, this study challenged poults before their natural nocturnal fast to determine the effects of supplementation on Salmonella challenge during grow-out. Three-week-old turkey poults, on a 2.5% lactose and Lactobacillus acidophilus (1x10^9 organisms/liter) drinking water supplement from day of hatch, were orally challenged with 1.7x10^8 nalidixic-acid resistant Salmonella enterica serovar Typhimurium strain 4232. Crop ingesta and tissue were collected at timepoints 0.5, 4, 8, and 24 hours post challenge. Crop ingesta and crop wall tissue were separately weighed and pulverized in a stomacher. Ten-fold serial dilutions were made in peptone water and plated on XLT 4 media. An immunohistochemical staining procedure was used to explore the effects of supplementation on invasion, clearance, and multiplication of Salmonella typhimurium in vivo. Results from this study indicate that lactose and L. acidophilus supplementation did not reduce S. typhimurium colonization after challenge.
Chapter 1. LITERATURE REVIEW

Introduction

Salmonella enterica serovar Typhimurium is a major source of gastrointestinal illness in the human populace with an estimated 1.4 million cases in the U.S. annually. S. typhimurium infections are predominantly caused (estimated at 96%) by consumption of contaminated food, with poultry meat implicated as the primary source (Mead 1999). Poultry consumption has reached record levels in this country so practical solutions to reduce Salmonella contamination in the bird are warranted. Foodborne infections are estimated at 33 million per year (45). Of those, Salmonella is the leader in food poisoning-related deaths, accounting for 31% (45). Total cases of foodborne salmonellosis may be underreported by 38 times (45) due to the fact that most people get ill and do not seek medical attention.

Salmonella typhimurium is a non-host adapted serotype because it can infect a variety of species, including cattle, swine, poultry, rodents, and man. Control of S. typhimurium is especially difficult due to its ubiquitous nature. Turkeys and chickens can carry S. typhimurium in their intestine without showing signs of disease and shed it in their feces. The crop of the bird is increasingly cited (9, 26) as a source for poultry meat contamination because it can break open during modern evisceration techniques and contaminate the carcass. Feed withdrawal has been used to reduce the incidence of fecal contamination on the carcass at slaughter but this practice leads to pH changes in the bird’s crop that can create a suitable environment for Salmonella growth.

The normal bacterial flora of the crop is protective against challenge with Salmonella. Techniques for maintaining the normal flora include 1) supplementing the diet of the bird to
maintain the normal flora and 2) giving the hatchling bird intestinal contents from a mature
bird to prevent enteropathogen invasion in the first place. Either one of these techniques
appears to be a feasible option in the production setting but each varies in efficacy.
The anatomy and function of the crop, normal flora present and how enteropathogens interact
with the alimentary flora, along with possible solutions for reduction of Salmonella
colonization and consequent food borne illness are reviewed.

**Crop Anatomy, Function, and Bacteria present**

The crop of the turkey and chicken is an expandable pouch in the esophagus, lined with
stratified squamous epithelium and mucous glands (47). Until recently the crop was thought
to have only a temporary food storage function (18). However, it is possible that the crop
may have other roles including the following: 1) a role in digestion with fermentation of
digesta, 2) potential to act as the first line of defense from harmful bacteria through
competitive exclusion, and/or 3) a possible immune function.
The crop surface structure microscopically resembles the bovine rumen (11) instead of the
previously thought “esophageal pouch”. The bacterial flora present are also morphologically
similar to those in the rumen (10) and have been shown to produce fermentation products
such as lactic, butyric, and propionic acids (11). The digestion of sugars due to amylases
(11,55), lactic acid bacteria species such as Lactobacillus (22), and Bifidobacterium (52) also
occur in the crop.

Chickens show a distinct daily variation in feed consumption with feeding restricted to the
hours of daylight with a marked increase in feed consumption in anticipation of the nocturnal
fast (12,61). The crop acts as a major mechanical storage site for energy and protein during
the night (12,61). As a turkey eats, the ingesta first pass directly through the crop (spending
less than one second there), filling the intestines and proventriculus (17,36). After the intestines and proventriculus become filled, the crop fills and takes on a storage role (17,36). The meal ends when the stretch receptors in the crop signal that the crop is filled. Crop emptying is mediated by an empty proventriculus or “stomach” in the turkey (17). The type of feed the turkey eats was thought to affect this pattern. Glucose-rich feeds do not induce satiety in the same way as less energy rich foods birds eat in the wild (36). It is not yet clear why this is, though increases in insulin in chickens and other species results in an increase in feed consumption (36). Medium chain triacylglycerols also retard chicken crop emptying, possibly due to rapid oxidation in the liver and disruption of signals from the intestine due to a ketone body by-product, β-hydroxybutyrate (24). This suggests that lipids may delay crop emptying, much like the effect they have in the stomach of mammals. Handling of birds itself reduces duodenal satiety signals in chickens due to stress hormones (36).

**Normal flora of the crop**

Bacteria are normally found in the gastrointestinal tract of animals. These bacteria aid in digestion and protect against the invasion of pathogenic bacteria. Lactobacilli are the most commonly documented normal flora species of the chicken and turkey. Lactobacilli are present in the crop (21) and the intestine (22) of domestic avian species. The *Lactobacillus* species present at one-day post hatch in the chicken remain for the bird’s entire life (20). These normal flora “inoculate” the ingesta to provide a suitable digestive environment in the intestine. Lactobacilli provide a bacteriostatic environment by lowering the pH to 4.5 in the chicken (20). Reduction of pathogens in the crop may lessen the possibility of colonization in the intestine (20). The addition of *Lactobacillus acidophilus* to the diet of chickens had similar effects to antibiotics with an increase in feed conversion (69) although which
Lactobacillus species are normally present in the crop of the bird and which are essential for reduction in colonization of pathogenic bacteria has not yet been defined. Techniques that initiate colonization by these normal flora in the young bird and their maintenance in the mature bird are reviewed in latter sections.

*Lactobacillus reuteri* supplementation was thoroughly explored in a 1997 study by Edens and colleagues (19). *In ovo* administration of *L. reuteri* was protective against *Salmonella typhimurium* challenge while not affecting hatching rates. The addition of *Lactobacillus reuteri* was safe and effective in both chickens and turkeys *in ovo and ex ovo* (19) in reducing susceptibility to enteric colonization by *Salmonella*.

Bifidobacteria have recently been described as beneficial bacteria in the chicken crop (52). Bifidobacteria were previously thought to only inhabit the ceca of the bird. Bifidobacteria, like lactobacilli, ferment glucose and fructose and are grown on similar culture media. These bacteria may prevent harmful bacteria from colonizing the crop. Since they are often associated with lactobacilli, their separate existence and properties have not yet been fully examined in the bird.

Birds decrease their population of flora when fasted (21) due to epithelial cell sloughing in the crop and intestine. Feed withdrawal prior to slaughter decreases fecal contamination of the carcass during slaughter procedures (44). The practice of feed withdrawal prior to slaughter has been cited as a major factor in *Salmonella* colonization, however, because this period of feed withdrawal causes a pH increase in the crop of the bird (26). This leads to changes in pH that create a condition more favorable to *Salmonella* growth and less favorable to resident lactic acid bacteria (14,34) that are protective against pathogenic challenge (13,15,31).
Crop contamination with *Salmonella*

When the resident flora of the crop become disturbed and are no longer able to protect against colonization, the crop can become a source of potential food borne pathogens. In 1995 Hargis and colleagues, showed that the crop of the broiler chicken was a major source of *Salmonella* carcass contamination in poultry processing plants due in part to the practice of feed withdrawal (26). This research, combined with previous work (34), found that not only is the crop a site of bacterial propagation, the neck of the carcass is the most often contaminated. Hargis and colleagues found that a *Salmonella enteritidis* challenge dose of $1 \times 10^6$ bacteria resulted in 30% of the crops remaining colonized with *S. enteritidis* two days post challenge. A challenge dose of $1 \times 10^8$ *S. enteritidis* resulted in 57% of crops still culture positive for *S. enteritidis* two days later and 37% were still positive at seven days after challenge (26). Survey data at a processing plant found that the chicken crop is 3.5 times more likely than cecal tissue to rupture upon evisceration (26). The crop tissue was also 85 times more likely to be contaminated with *Salmonella* than the ceca (26). This research revealed that studies that concentrate only on intestinal *Salmonella* colonization as a source of carcass contamination could be focusing on the wrong end of the bird and that methods were needed to control crop bacteria as well.

**Impact of *Salmonella* infections in the human population**

It is difficult to estimate how much contaminated crops contribute to food borne illness in the human population. *Salmonella typhimurium* infections in the United States have increased since mid-century (49). After the near-elimination of *Salmonella typhi* as a disease threat due to sanitation improvements, other non-typhoidal *Salmonella* serotypes have gained in prominence (49). Eradication programs in the United States have considerably decreased the
incidence of poultry salmonellosis caused by poultry adapted serotypes such as Gallinarum (Fowl typhoid) and Pullorum (Pullorum disease) in the United States (64). These diseases cause production losses so an agglutination test exists for screening birds for Gallinarum and Pullorum infection (60). In the period between 1987-1997 *Salmonella typhimurium* was the most common of the 2449 known *Salmonella* serotypes isolated from humans (49). Since *S. typhimurium* is a non-host adapted strain, it passes easily from a large range of hosts.

Ninety-six percent of *Salmonella typhimurium* infections in humans are attributed to the consumption of contaminated food, with poultry products cited as a major source. Overall, *Salmonella* related food poisoning causes 1.4 million cases and 5000 deaths in the US annually (45). Estimations of medical care costs and lost work productivity from *Salmonella* amounted to about $3.5 billion for 1993 (25,70).

*Salmonella typhimurium*, though implicated in egg contamination when it emerged as a foodborne pathogen in the late 1960's, failed to infect eggs either through the hen's ovary (yolk contamination) or the shell from fecal contamination (5). Later work did show that *Salmonella typhimurium* is capable of infecting ovarian tissues but its survival inside the egg to cause disease is not as great as *Salmonella enteriditis*. This is indicated by the near non-existence of reported egg and *Salmonella typhimurium* foodborne illness cases (40).

Antimicrobial resistance is of additional concern when considering the prevalence of *Salmonella typhimurium* in human and livestock populations. Antibiotic use in the livestock population has allegedly led to multiple antibiotic resistant food borne pathogens in the human populace (68). Infections that result from anti-microbial resistant organisms tend to have a longer convalescent period, making the course of illness more severe and will result in more morbidity (68).
Preventing or reducing colonization initially for non-host adapted strains may be the best solution for control in the avian flock since eradication is currently not an attainable solution.

**Salmonella Pathogenesis in Poultry**

*Salmonella typhimurium* attaches to the crop epithelium (59) of the chicken and it is thought that as the epithelium sloughs off it is carried down to the intestine. *Salmonella enterica* serovar Typhimurium is a facultative intracellular pathogen, replicating in macrophages. Identification of the over 2500 serotypes involves examining differences in the O (agglutinating antibody based on the lipopolysaccharide or LPS outer membrane) antigen and the H or flagellar antigen according to the Kauffman-White method (6). Pathogenesis of *Salmonella* infection in the chicken varies according to *Salmonella* strain encountered, with *Salmonella typhimurium* causing rapid inflammation upon entry into intestinal epithelial cells by way of a type III secretion system which ilicits a large inflammatory response (39). The resulting diarrhea and sequelae are a result of an overactive immune response involving neutrophils in humans and in the avian species, the equivalent heterophil (29,76). The immune response in susceptible chickens resembles that of humans (39).

To further illustrate the effects of *Salmonella typhimurium* challenge in the chicken, specific pathogen free chicks were used (59). *Salmonella typhimurium* was isolated from crop and cecal tissue at seven and 28 days (59). Out of the 14 serotypes of *Salmonella* tested, the highest percentage of mortality was caused by *S. pullorum* followed by *S. typhimurium*. The same pattern was true for reduction in body weights at days 7, 14, 21, and 28. Birds challenged with *S. typhimurium* had severe fibrinosuppurative inflammation of the pericardium and mild hepatitis, peritonitis, and mild lymphocytic pneumonia (59). Most
pathologic lesions associated with *S. typhimurium* challenge were consistent with those of *S. pullorum*, a host adapted strain (59).

Post-hatch chicks challenged with $1 \times 10^8$ *Salmonella typhimurium* had varying rates of mortality, depending on *Salmonella* serotype and breed of chicken (7). Susceptible chicks had diarrhea, dehydration and lethargy, and ultimately died. The highest level of bacteria recovered were found in the spleen, liver, and ceca (7). *Salmonella typhimurium* has high fecal excretion rates for a shorter duration of time compared to other food-pathogen *Salmonella* strains (8). Younger chicks have longer excretion times than older chickens, indicating that a mature alimentary flora and macrophages are important for resistance and clearance to infection (8). If exposure to *Salmonella* can be avoided during the first few days of a chicken or turkey's life it appears that the bird will be resistant.

**Flock *Salmonella* epidemiology**

The best possible defense against possible food borne pathogen colonization would be the absence of undesirable bacteria in the environment. This is a remote possibility in the case of a relatively ubiquitous organism like *Salmonella typhimurium*. Salmonellae can be found in feed (27,1), litter (15), rodents (72), other birds, and perhaps even on the human caretakers (4) while the poultry are growing out. Aerosolized *Salmonella* can survive for as long as 90 minutes (72) and *Salmonella typhimurium* can persist in feed for up to 16 months and in litter for 18 months at 25°C (25). Maximum intestinal *Salmonella* colonization occurs at the second or third week of life for broilers and persists in decreasing levels until slaughter. *Salmonella* infections in a poultry flock can persist for 98 days in cecal tonsilar tissue (42) and can be detected by serum agglutination for 42 days (60).
Susceptibility to *Salmonella* infection in chickens is heightened by many factors: 1) age, 2) bacterial survival through gastric barrier passage, 3) effective competition with other bacteria, 4) location of a hospitable colonization site, 5) diet, 6) physiological status, 7) health and disease status, 8) environmental stresses, 9) medication effects, and 10) host genetic background (3).

During production these factors make exposure to *Salmonella* prior to slaughter almost inevitable. Stress caused by transport to harvest makes chickens especially vulnerable to pathogen colonization, which can lead to food safety concerns.

**Detection of Salmonella in the poultry flock and at processing**

The location on the chicken carcass where the *Salmonella* contamination is found and the appropriate method for sampling and surveying possible *Salmonella* contamination varies. In a study published in 2002, raw, whole, retail-purchased chickens were surveyed in England from 1998 to 2000. Their procedure used neck skin, whole carcass rinse, carcass-rinse with remaining skin attached, exterior packaging swabs, and whole packaging rinse. Two different media for *Salmonella* enrichment were also compared: Rappaport-Vassiliadis soya (RVS) peptone broth and selenite cystine broth with sodium bioselenite (SCB). The RVS gave higher recovery rates, 34% vs 20% for neck-skin samples and 24% vs 18% for entire packaging. Overall, samples most often contaminated with *Salmonella* were neck-skin (26%) and carcass rinse plus neck skin (25%). Examples of serotypes isolated were Hadar (28%), Enteritidis (16%), and Typhimurium (3.3%) (38).

The advent of polymerase chain reaction (PCR) detection methods for *Salmonella* contamination is thought to improve sensitivity of carcass contamination screening methods (74). Neck skin samples were collected aseptically and subjected to a typical culture
methods: homogenized in peptone water, enriched in RV broth, and then plated onto brilliant green agar (BGA) for enumeration. The PCR method detected a 19% contamination prevalence while the culture method found 16% (74). Two reported drawbacks from traditional culture methods compared to PCR were noted in this study: 1) time: 4-7 days to culture samples vs 24-30 hours to perform PCR and 2) decreased sensitivity of the culture method. PCR can detect fragments and non-viable bacteria that are not detectable in culture methods. (These injured or dead bacteria do not cause disease.) The PCR procedure failed to detect *Salmonella* on 7 samples that the culture method found to contain *Salmonella* and this was not accounted for by the authors. When combining both methods, 23% of neck skin samples were found to be positive (74). This study reiterates the fact that the neck of the carcass is the most often-contaminated area suggesting that crop contamination is an important source of food borne pathogens.

Prevention of *Salmonella* colonization in turkey prior to processing, begins with testing the live bird to determine if the bacteria are present. Methods for detection of *Salmonella* infection within the turkey flock have been compared (60). Serum agglutination tests, though varying in detection rates by test, were more accurate than cloacal swabs to indicate infection. IgM response detected by microantiglobulin (MAG) and IgG detected by microagglutination (MT) provides a reliable method, according to a 1984 report (60). ELISA testing has been introduced to examine antibody responses to *Salmonella* infection. Challenge, then re-infection with *Salmonella typhimurium* in chickens (28) revealed that when challenged at four days of age, chickens shed bacteria for up to 10 weeks. When the same chickens were exposed to the bacteria a second time after 10 weeks, they excreted it at a lesser rate, showing that age of the chicken makes a difference in persistence of infection.
Salmonella Control Techniques

Preventing *Salmonella* from colonizing the digestive tract of chickens or turkeys can be accomplished by the establishment and maintenance of favorable intestinal flora. Probiotics are live bacteria provided for that purpose and prebiotics are dietary supplements given to provide nutrition for the normal flora—such as lactose or lactic acid. The ability of dietary supplements in the feed or water to decrease crop contamination has been examined. The addition of 2.5% lactose in the water of broilers prior to slaughter did not “consistently reduce crop or cecal colonization levels” (9). The chickens in that study were provided the lactose 5 or 11 days prior to and throughout an extended 18 or 24 hour feed withdrawal period. The addition of 5% lactose to an anaerobic culture treatment did not aid in reduction of *Salmonella* recovery from the ceca of turkeys as it did for chickens (33). Corrier and others (14) found that 5% lactose in the diet reduced *Salmonella seftenberg* colonization in the ceca of turkey poults.

More recently, the addition of 0.44% lactic acid to the water reduced the number of *Salmonella* positive crops in broiler chickens at slaughter. These chickens were subjected to 8 and 12 hour feed withdrawal periods (13). The addition of acetic or formic acid also reduced the incidence of *Salmonella* positive crops. The administration of acids in the water did reduce water consumption however, which could create production problems, such as a drop in feed consumption.

Pathogen-reducing merits of sucrose- vs glucose-based water supplements during feed withdrawal in broiler chickens has been compared as well (31,51). Supplementation of D-mannose in the chicken diet reduced intestinal colonization with *Salmonella typhimurium* (51). Sucrose levels from 2% to 8% reduced *Salmonella* recovery in crops whereas 10%
sucrose and glucose were not effective (31). It is hypothesized that supplements given in water during the feed withdrawal period are only useful for reducing enteropathogen numbers when they are given at concentrations that the crop resident lactic acid bacteria can use (31). Any residual carbohydrate substrate not taken up by the resident flora is left for use by problematic bacterial invaders.

Bacteriophages have also been proposed for *Salmonella* control. An effective phage may be difficult to isolate (2). Bacteriophage administration just prior to slaughter to eliminate *Salmonella* could conceivably reduce bacterial counts in the crop, small intestine, and ceca (2). As of this time, the approach has not been developed. The rapid mutation and diversity of *Salmonella* serotypes may prohibit the practical use of bacteriophages.

**Competitive exclusion**

Another approach to *Salmonella* control may be as simple as dosing the chick with intestinal contents from a mature bird. Nurmi cultures are intestinal cultures from a mature bird given orally to a chick to prevent attachment of potentially harmful bacteria (57). Since the gut of the chick is sterile when it hatches, the cecal culture provides an intestinal flora for the chick free of undesirable bacteria. This accomplishes the following: 1) occupies potential invading bacteria’s binding sites on the alimentary tract wall, 2) uses sugars needed for nutrition for invading bacteria, and 3) maintains a pH in the tract that supports only resident bacteria. This procedure, first used by Nurmi and Rantala in 1973 in chickens, is termed competitive exclusion (57).

A similar effect has been observed in turkeys. In fact, when inoculated with a fecal preparation from a mature chicken, turkey poults were protected from *S. infantis* and *S. typhimurium* challenge (65). Cecal cultures given to poults from the adult turkey were also
able to protect against *S. typhimurium* colonization (43). Day of hatch turkey poultets were more resistant to *Salmonella typhimurium* challenge, when compared to non-treated controls when they were given adult turkey intestinal cultures. The protection varied by challenge dose (58). *Salmonella typhimurium* challenge dosages of greater than $10^6$ caused infection but less bacterial fecal shedding than in untreated poultets, even when administered up to 20 hours post-challenge (58). Turkey- or chicken- derived Nurmi cultures offered protection against *E. coli* O157 and *S. typhimurium* challenge in the turkey (73). Results from undefined competitive exclusion (CE) cultures in turkeys have been conflicting (32,33). The bacteria present in a successful CE culture for a turkey must contain anaerobic bacteria that produce the volatile fatty acid by-product propionic acid (32), regardless of turkey or chicken origin. None of the above work investigated the impact of CE on the crop of the bird as opposed to the ceca so the value of CE cultures and their influence on crop flora is relatively underinvestigated.

It is hypothesized that mucosal competitive exclusion cultures may offer more protection than the traditional CE culture. Mucosal competitive exclusion cultures contain more cecal epithelial tissue and were originally targeted to reduce *Campylobacter* colonization. These cultures decreased the *Salmonella* colonization rate by almost half as compared to cecal content CE alone (66).

Procedures involved in the development of a CE culture were reviewed in 1992 (67). Problems in CE culture development and use include the large number of microbes present in the ceca of the bird, storage difficulties causing the loss of potency, lack of consistent protection against challenge, and a general lack of knowledge of the mechanisms involved in resistance to intestinal colonization. Since the publication of this report, undoubtedly
advances have been made, although the amount of CE culture to use, the best method for providing it, and how other agents interfere or enhance the treatment’s efficacy are an ongoing research area.

Protection from enteropathogens is reduced as the dosage of a CE culture is decreased. Protection from pathogen colonization depends on exposure conditions. A study by Corrier et al (16), notes that at a *Salmonella* challenge dose of $1 \times 10^4$ CFU/ml the CE culture gave the best protection at $1 \times 10^{11}$ CFU/ml.

The development of defined competitive exclusion cultures, (bacteria identity known and reproducible) which contain the ideal balance of *Lactobacillus* and lead to the production of propionic acid may be the most beneficial for turkey production. Some CE cultures are becoming available in the US, though the presence of pathogens is also of concern when using a continuous, laboratory-raised, CE culture, especially the possibility for passage of antibiotic resistant organisms (71).

Another potential problem in the use of CE cultures in the turkey flock lies in applying research done in chickens to the turkey. The normal turkey crop maintains a pH that differs from that of the often-cited chicken. The crop of the turkey is at a pH of 6 whereas the chicken maintains a pH of 4.5 (18). It is unclear if this difference has an effect on *Salmonella* colonization and if comparisons between the species can be assumed.

**Pre and probiotic supplementation for *Salmonella* control**

Probiotics are live bacteria consumed to change the composition of the intestinal flora whereas prebiotics are substrates (usually milk sugars or oligosaccharides) that feed the beneficial flora. Probiotic bacteria that are presumed to be harmless are ingested to replace existing, more inefficient or potentially harmful bacteria (50). Suspected benefits of
probiotics in humans include increased immunity from enteropathogens, treatment for allergic or autoimmune diseases, elimination of lactose intolerance, and decreased risk for irritable bowel disease and colon cancer (50).

Fructooligosaccharides are prebiotics that consist of one to three fructose molecules attached to a sucrose and are thought to enhance the growth of intestinal flora such as *Lactobacilli*. The use of prebiotics, such as fructooligosaccharides (FOS), and their effects on the intestinal flora of chickens by themselves or in combination with CE cultures has been investigated. In 7 and 21 day old chicks the addition of 0.1% FOS to the diet from day of hatch, a reduction in *Salmonella enteritidis* colonization was noted, though more effectively for 21 day old chickens (23). When the 0.1% FOS supplement was combined with CE, the number *S. enteritidis* per gram of cecal content was reduced and tissue invasion was inhibited (23).

Antimicrobials are commonly used in poultry feed in the U.S. for growth promotion and prevention of disease (68). The use of antibiotics and their influence on the effectiveness of CE cultures is a concern if CE cultures are used in the production setting. In laying hens after molt, the feed withdrawal period can render hens susceptible to salmonellae (63). Since layers possess a mature intestinal flora, CE cultures have not been an effective option for *Salmonella* control during normal production (63). During molt however, a cycle of antibiotics followed by a CE culture has been shown to decrease the incidence of egg contamination and eliminate the need to slaughter hens instead of reusing them for another cycle (63). This procedure was previously investigated with the use of a commercial CE treatment after antibiotic administration in the feed or water (37). Antibiotics given in the feed were more successfully administered compared to those given in the water, especially
when combined with CE. These treatments, while effective at treating *Salmonella* colonization raise the issue of antibiotic resistance and impending restrictions on antibiotic use (63).

Aflatoxins may have a role in competitive exclusion effectiveness. Aflatoxins are present in grains and are toxic to animals, birds, and people at high doses (41). They can decrease volatile fatty acid (VFA) concentration in the ceca thus affecting poultry growth at lower doses. Aflatoxins, specifically the T-2 toxin when present at significant concentrations may also render the bird more susceptible to *Salmonella typhimurium* even if given a CE culture. This may be due to loss of anaerobic flora or irritation of the gut caused by the toxin itself though the exact mechanism is unclear at this time (41). Environmental factors, such as the presence of antibiotics and aflatoxins in poultry feed are valid concerns for the effectiveness of the CE culture in the production setting. Commercially available CE cultures in the US must be tested for use in the field as well to ensure their usefulness.

Defined CE cultures are commercially available in other countries and have been for decades. Their use has been proven to reduce *Salmonella* colonization in chicken flocks (30). In the US, recently PREEMPT™, which is a defined culture of 29 isolates of bacteria, has been made available for use and submitted for approval to the FDA (48). Bayer offers a partially defined normal avian gut flora (NAGF) product called Avigard™ that is lyophilized (63). Other products such as Broilact™ have been used in Sweden and Finland (67).

**Use of *Salmonella* Vaccines**

Combining vaccination with CE might be suggested as the ultimate option for *Salmonella typhimurium* control. Recent research supports this theory (46). Study birds were “vaccinated” with a wild type live strain of *S. typhimurium* and given CE cultures either
simultaneously, vaccinated first, or vaccinated after the CE administration. When given the vaccine, followed by the CE culture, protection from challenge exceeded that of either treatment alone. High doses of the vaccine, and a possible boosting dose, are required however (46). Secretory IgA has been detected in the chicken crop after *Salmonella enteritidis* challenge (62). Secretory IgA levels were highest in response to flagellar preparations of *Salmonella enteritidis* (62). Vaccines which target the IgA mucosal response against *Salmonella* flagella in the crop may be an option for prevention of colonization in the intestine.

The use of vaccines for *Salmonella* control, their efficacy, and current research was reviewed recently (75). Live, attenuated 9R *S. gallinarum* vaccine has been in use since the 1950’s. Since the commercial eradication of Fowl typhoid as a disease threat in poultry, this vaccine has declined in use. Some cross-protection between *Salmonella* strains is conferred and many genetic mutants exist for vaccine use poultry, though their efficacy varies (75). Most vaccines reduce fecal shedding or disease in chicks (75). Methods of administration for non-host adapted live Salmonella vaccines, the feasibility of vaccinating chickens with live *Salmonella*, and the associated costs are still under investigation (75).

**The Poultry Industry**

Poultry consumption in the United States has increased during the last 30 years. Poultry meat is now 34% of the total meat consumed (1997). This represents 31 pounds more poultry than consumed than in 1970. The amount of turkey consumed has doubled- 6.5 pounds a year in 1975 to 14 pounds a year in 1998 (56). This trend is driven by the popular opinion that poultry is a more consumer-friendly and affordable product than beef or pork. The emergence of ready-to-eat products, consistent product, and more extensive marketing of
“healthy” cuts, such as breast meat, have led to higher amounts of poultry meat consumed than ever before. With the increasing demand for poultry products, it is imperative that food safety issues be addressed in a timely manner.

Vertical integration, a reduction in the numbers of producers and processing plants in the poultry industry makes it easier to introduce changes in production than in other meat industries, as long as they are cost effective. A 1992 Swedish study weighed the benefits vs the cost of competitive exclusion and concluded that if the CE concept can be applied in a widespread manner, Salmonella food contamination could be reduced by 94% (53). The cost associated with implementing CE as opposed to making changes in automated slaughter procedures or more widespread sampling and monitoring of food products is a relatively affordable. CE has been effectively used in Finland since 1976 and Sweden since 1981 (30). There was a Salmonella incidence of just 2.6% in 2068 broiler flocks in Finland in 1990 (30). Salmonella-free turkeys can be produced under controlled production practices (54). A large-scale USDA study showed that if Salmonella-free pouls are provided to the grower, if rodents are exterminated, and feed in the barn is Salmonella-free the birds will remain uncontaminated. The feed used in that study was pelleted and free of animal by-products which have been implicated as a source of Salmonella.

Conclusion

The control of food borne pathogens in poultry production is a daunting task. With the variety of factors that influence colonization of the alimentary tract of chickens and turkeys, it may not be possible to develop a simple solution. Increasing research into the immunologic mechanisms involved in clearance of Salmonella infections in poultry and knowledge of what is required to maintain the infection have shed light on some aspects.
Typhimurium can enter the flock from rodent and feed sources during grow-out and colonize the digestive tract just prior to slaughter during a feed withdrawal period. The crop of the bird is a thin-walled structure in the neck of the bird that is vulnerable to breakage in evisceration processes, which can contaminate the carcass. Feed withdrawal, which is intended to clear the bird’s intestine of fecal material prior to slaughter induces pH changes in the crop that can lead to an environment more conducive to Salmonella colonization. Pre-harvest solutions to reduce the rate of Salmonella contamination and stabilize the pH of the digestive tract include competitive exclusion and pre- and probiotic supplementation that maintain the normal flora present. Competitive exclusion has been used in other countries for years, but complications in storage and defining content of the culture have delayed their widespread use in the US. Techniques to reduce the incidence of Salmonella typhimurium contamination of poultry are a priority for the expanding poultry industry and may be as simple as a feed additive.

The following study uses a lactose and Lactobacillus acidophilus supplement in the drinking water of three-week-old turkeys to determine if pre and probiotic supplementation can reduce Salmonella typhimurium contamination in the crop. The effect of Salmonella typhimurium colonization and the use of supplementation on crop emptying, colonization on the crop and crop contents, and histological effects of bacterial colonization are used to show differences between treated birds and controls.
Chapter 2. MATERIALS AND METHODS

Animals and Husbandry

One-day-old male Hybrid turkey poults were obtained from Midwest Hatcher (Dike, Iowa). The seventy-two poults were assigned to three batteries split according to treatment (see below). Daylength was reversed to facilitate sampling during the subjective “night”. The lights were turned off at 10 AM and came back on at midnight. (14 L:10 D). All poults received an unmedicated game bird ration (Purina Game Bird crumbles 30% crude protein, 5.5% fiber, and 2.5% fat ) and provided water ad libitum.

Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>Challenge</th>
<th>Water Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>no</td>
<td>none</td>
</tr>
<tr>
<td>B</td>
<td>yes</td>
<td>none</td>
</tr>
<tr>
<td>C</td>
<td>yes</td>
<td>2.5% Lactose and <em>Lactobacillus acidophilus</em></td>
</tr>
</tbody>
</table>

The day of arrival, poults in group C started receiving 2.5% lactose (Sigma St. Louis, MO St. Louis, MO) and (1x10^9 per liter) *Lactobacillus acidophilus* (Thomas Laboratories Tolleson, AZ) in the water. Five days prior to *Salmonella typhimurium* challenge, poults were moved into cages grouped by treatment and timepoint with six poults per cage.

*Salmonella Screening*

At one week of age, the turkey poults were screened for *Salmonella* by vent swab enrichment and culture using the method of Hurd, *et. al* (35). Individual birds were identified by wing band at this time. The swab was placed in GN Hajna (Difco Laboratories Detroit, MI) broth at 37°C for 4 days, then transferred to Rappaport-Vassiliadis broth (Difco Laboratories...
Detroit, MI) for 24 hours, and finally streaked onto XLT₄ plates (Difco Laboratories Detroit, MI). All turkeys were negative for *Salmonella*.

**Challenge Method**

Nalidixic acid resistant *Salmonella enterica* serotype Typhimurium strain 4232 (kindly donated by Irene Wesley at the National Animal Disease Center, Ames, IA) was grown overnight on blood agar plates (5% bovine blood, Difco Laboratories Detroit, MI Blood Agar Base). A swab of this plate was inoculated into pre-warmed Trypticase Soy Broth (Difco Laboratories Detroit, MI) and grown with agitation at 220 RPM on a shaker at 37 °C for one hour and 15 minutes. The culture was diluted to an optical density of 0.35 at 595 nm (Spectronic 20). The challenge dose was determined to be $1.7 \times 10^8$ cells/0.5 ml dose by serial dilution and plate counts. The turkeys were challenged orally at the end of the light period.

**Sample Collection and Assay Procedure**

The concentration of *Salmonella* in the crops was determined in the following manner:

Crops were aseptically collected into individual sterile plastic bags immediately after the birds were euthanized by cervical dislocation. Time points for post-challenge harvest were 30 min, 4 hours, 8 hours, and 24 hours. Six birds per timepoint for each group were used. Crop weights, including content for each poult, were taken. The content was then aseptically removed and a representative sample, about 1.5 ml was retained. An approximately 2 cm² section of the crop wall was fixed in buffered formalin. The remainder was rinsed in peptone water (Difco Laboratories Detroit, MI) and weighed.
Both crop wall and crop content samples were diluted to a ratio of 1 g sample:2 ml peptone water and then homogenized in a Stomacher (Seward Stomacher 80) blender. Crop content samples were pulverized for two minutes and crop wall for one minute.

**Salmonella Quantification (Colony Forming Unit Determination)**

The number of colony forming units of *Salmonella typhimurium* for the resulting homogenized suspension for treatment groups B and C was determined. Samples were diluted as follows: serial 10-fold dilutions of crop and crop content were made to $10^{-6}$ in microtiter plates and then 10 µl of each dilution was streaked across three replicate XLT$_4$ plates (Difco Laboratories Detroit, MI) containing 50µl/ml Nalidixic acid.

In addition, one ml of each crop and crop content homogenate was placed into fifty ml of Tetrathionate broth (with 50µl/ml Nalidixic acid) for enrichment. If the sample was found to be negative for colony forming units after overnight incubation on XLT$_4$ plates (containing 50µl/ml Nalidixic acid) the tetrathionate enriched sample was passed through 2 RV enrichment steps and plated again on XLT$_4$ to see if any *Salmonella* were present.

**Antisera to *Salmonella typhimurium***

After testing several commercially available antisera to *Salmonella* for use in immunohistochemistry, it was decided that sensitivity could be improved with the production of antisera directly to the challenge strain used (4232).

*Salmonella typhimurium* was grown on a Mueller-Hinton agar plate for 18 hours at 37° C. The bacteria were rinsed from the plate with 0.9% NaCl. The bacteria were pelleted, resuspended, and washed twice with saline. An aliquot of the bacterial suspension was streaked onto blood agar plates to ensure purity. The bacteria were heat-killed at 60°C for
one hour after which the suspension was streaked onto MacConkey’s and blood agar plates to make sure it was no longer viable. Optical density readings indicated that the concentration of *Salmonella* in the vaccine was $1 \times 10^8$ cells/ml. Five percent aluminum hydroxide resorbine (Intergen, Norcross, GA) gel was added as an adjuvant. Two New Zealand white rabbits (Harlan-Sprague Dawley) were injected at three times at two-week intervals. Rabbits were injected with 0.5 ml at each of two subcutaneous sites. Antibody production was monitored by a standard plate agglutination test using strain 4232 as antigen.

Antiserum was tested for cross-reactivity with organisms found in the normal turkey crop. The antiserum reacted with *E. coli* in the immunohistochemical staining procedure. To eliminate this cross-reactivity, the antiserum was absorbed against the strain of *E. coli* recovered from the crop. The *E. coli* was grown overnight on bovine blood agar plates at 37°C and washed from the plate with 0.9% saline. The *E. coli* suspension was centrifuged to pellet and washed 3 times in saline. The resulting suspension was combined with the antiserum for an overnight incubation at 4°C. The supernatant was centrifuged for 10 minutes at 14,000 rpm to remove any bacterial debris. The antisera were preserved with 0.01% sodium azide (Sigma St. Louis, MO St. Louis, MO). These procedures, along with the use of the Dako Envision kit that does not include an animal-generated secondary antibody but instead a polymer increased the sensitivity of the assay.

**Immunohistochemistry**

To examine colonization and clearance over time of the *Salmonella typhimurium* in the crop tissue immunohistochemistry was performed. All of the following histopathologic procedures were done in the histopathology section of the Iowa State University Veterinary Diagnostic laboratory. Automated equipment (such as coverslipper and slide washing
system) the was used as often as possible to ensure consistency and attempt to make
Salmonella typhimurium staining applicable to the diagnostic setting. Formalin-fixed crop
tissue from turkeys was embedded in paraffin and cut at 4 microns. For
immunohistochemistry (IHC) the slides were dried at 60°C for 20 minutes and then de­
paraffinized by three, 5 minute changes in Propar™ (Anatech, Ltd. Springfield, VA) and
hydrated in distilled water.
The slides were then quenched in 3% hydrogen peroxide for 10 minutes to remove blood
products. After two rinses in distilled water, a 0.05% protease (type XIV) (Sigma St. Louis,
MO) digestion was performed for 2 minutes at room temperature. The E. coli cross-absorbed
rabbit primary antibody was diluted 1:100 in antibody buffer and two drops were added to
each slide for 40 minutes at room temperature.
The Dako Envision™ system for IHC staining was used for the remainder of the steps: three
drops anti-rabbit polymer for 10 minutes, rinsed with Trisma-HCl pH 7.6 (Sigma St. Louis,
MO) buffer and then DAB substrate was added for 5 minutes then a rinse with deionized
water. The slides were then dehydrated in ethanol and cleared in Propar and coverslipped
using the automated Shandon coverslipper which affixes with Histomount™ (Thermo
Shandon Pittsburgh, PA).

**Hematoxylin and Eosin Staining**

To further evaluate specificity of Salmonella typhimurium IHC staining, hematoxylin and
eosin (H&E) staining was used. The H&E method is a standard procedure and gave an
indication of the number of bacteria present in the crop. The H&E staining was performed in
the histopathology section of the Iowa State University Veterinary Diagnostic Laboratory.
Automated equipment was used as often as possible to ensure accuracy and repeatability.
For H&E the slides (see sectioning procedure above) were dried at 60°C for 20 minutes and then de-paraffinized by three, 5 minute changes in Propar™ (Anatech, Ltd. Battle Creek, MI) and hydrated to distilled water. Slides were stained in Gill’s Hematoxylin (Anatech, Ltd. Battle Creek, MI) for 30 seconds then rinsed in running tap water until the water ran clear, about five minutes. The slides were dipped 10 times in 95% ethanol, stained in one percent alcohol Eosin-Y (Richard-Allen Scientific Kalamazoo, MI) for 10 seconds and then dipped 10 times in 95% ethanol. The slides were then dehydrated in 100% ethyl alcohol 3 times at 10 dips each, cleared in Citrisolv (Fisher, Pittsburgh, PA) then in Propar three times. The slides were then coverslipped using the automated Shandon coverslipper which affixes with Histomount™ (Thermo Shandon Pittsburgh, PA).

**Statistical Methods**

Data analysis was performed via SAS PROC GLM (Carey, NC) on log$_{10}$ bacterial counts. Two-way analysis of variance was utilized to identify interactions between timepoint and treatment. Where an interaction was noted in the SAS analysis, a Tukey-Kramer multiple comparisons test was performed.
Chapter 3. RESULTS

Three-week-old turkey poults in treatment groups B (no supplements) and C (receiving lactose and *Lactobacillus* in the drinking water) were challenged with *Salmonella typhimurium* \((1.7 \times 10^8 \text{ cells in 0.5 ml})\) by oral gavage. Tables 1 and 2 show the number of *Salmonella typhimurium* recovered from, respectively, the crop contents (ingesta) and crop wall. The dose of *Salmonella typhimurium* administered was very similar to the aggregate *Salmonella typhimurium* number recovered in the crop wall \((4.4 \times 10^7 \text{ B, } 3.09 \times 10^7 \text{ C})\) and ingesta \((5.28 \times 10^5 \text{ B, } 9.11 \times 10^5 \text{ C})\) according to culture methods at the 30 minute time point. After 24 hours, the numbers of *Salmonella typhimurium* in the crop ingesta were decreased \((p < 0.05)\) to less than 1% of those that at the 30 minute time point after challenge in poults in both treatment groups B and C (table 1). The crop was empty \((< 1 \text{ g ingesta})\) at the 8 hour time point so it was not possible to make comparisons to crop contents at that time point.

No changes in the number of *Salmonella typhimurium* in the crop wall were observed between 1/2 and 8 hours following challenge (table 2). However, by 24 hours, crop wall *Salmonella typhimurium* numbers were decreased \((p < 0.05)\) to < 1% of those at the 0.5 hour time point in poults in treatment group B and to 1.7% in treatment group C by four hours.

There were no differences between treatments B and C in the number of *Salmonella typhimurium* in the crop ingesta or crop wall 0.5, 8 and 24 hours following challenge (table 1 and 2).

Table 3 summarizes the quantity of ingesta in the crops of the three treatment groups at the sampling times. The crops were engorged with feed at the beginning of the subjective night
in all treatment groups. The amount of ingesta remaining in the crop declined \((p < 0.05)\) by 4 hours on the poults of treatment group A (unchallenged) and was essentially gone \((< 1 \text{ g})\) from the crop by 8 hours in all treatment groups. There were no differences between the weights of the ingesta/crop contents with time and in the different treatment groups except 24 hours following \textit{Salmonella typhimurium} challenge. At that time point, there was lower \((p < 0.05)\) crop contents in the poults of group C than A with those of group B intermediate. This may reflect reduced feed intake following \textit{Salmonella typhimurium} challenge. Changes in the quantities of ingesta are similarly observed if the weight of the ingesta plus crop are considered (table 4). There were no differences with either time or treatment in the weight of the crop wall (table 5).

No reduction in crop contamination with \textit{S. typhimurium} was observed with the supplements used in this study. \textit{Salmonella} recovery was higher \((p<0.05)\) from supplemented poults, especially in crop content samples. The largest difference was at the 4 hour timepoint with 1.87 log higher \textit{S. typhimurium} recovered from the supplemented poults.

Immunohistochemical (IHC) staining of the sectioned crop showed \textit{Salmonella typhimurium} attached to both ingesta and crop wall. It appeared that the \textit{Salmonella} adhered to the crop wall was also in association with feed. The epithelium was sloughing off in both control and challenged poults and \textit{Salmonella} may have colonized the tissue of challenged groups B&C and then sloughed off over time and traveled to the intestine. No apparent differences in crop colonization were noted between the treatment groups with the IHC procedure because it was not a precise quantitative measure of colonization compared to culture methods. \textit{Salmonella typhimurium} staining did appear to decrease over time however. There were many other bacteria present along with the \textit{Salmonella typhimurium} challenge in the turkey crop. Since
IHC is not typically used in crop sections (or poultry diagnostics) it was difficult to determine the identity of the other bacteria present.
Chapter 4. DISCUSSION

Use of various supplements such as carbohydrate solutions, lactic acid, lactose, or Lactobacillus bacteria during the grow-out period may prevent or lessen Salmonella colonization of the young chicken or turkey. Supplements such as these in the domestic fowl diet are thought to propagate a favorable intestinal environment that is protective against colonization of invading bacteria by occupying space on the intestinal surface, using available nutrients, and maintaining a pH that is not ideal for pathogen growth (9,13,14,19,31). Lactic acid (0.44%) during the preharvest feed withdrawal period has been previously shown to reduce Salmonella contamination (13) in chickens. Lactose administration in chickens and turkeys prior to slaughter has reduced Salmonella colonization in some studies though results have been inconsistent (9,33,14). These studies, with the exception of one (9), examined Salmonella colonization in the intestine and did not consider the crop of the bird.

Administration of lactobacilli to chickens has had effects similar to antibiotics in previous studies (19) in growth promotion and resistance to infection. Giving the bacteria itself is thought to initiate and maintain a healthy gut microflora. Combining lactose supplementation with administration of lactobacilli may provide continuous supplementation to lactic acid bacteria in the avian gastrointestinal tract.

The present study utilized 3-week-old turkey poultts challenged with Salmonella typhimurium to explore any benefit of a 2.5% lactose and a commercially available Lactobacillus acidophilus addition to the water. This study was performed under reversed daylength so a natural feeding pattern, feed consumption during the day and nighttime
fasting, could be observed. By using this approach, natural challenge conditions were mimicked and the effect of *Salmonella* challenge and pre-harvest supplementation on crop emptying could be observed. Pouls were challenged 30 minutes prior to the dark cycle, during the period of their pre-darkness increase in feed intake. Crops were collected at timepoints 30 min, 4 hr, 8 hr, and 24 hour post challenge. The 24 hour timepoint allowed the pouls to feed one day post-challenge. Time points were similar to previous studies where feed withdrawal was induced (9,13,31), except the addition of a 24 hour timepoint that allowed refilling of the crop and further observations on *Salmonella* clearance over time.

The 2.5% lactose and *Lactobacillus acidophilus* supplement administered to a group C in the water did not reduce *Salmonella typhimurium* colonization in the turkey crop. In fact, in this work, pouls that were challenged but not given the lactose/lactobacilli had lower numbers of *Salmonella* bacteria recovered from crop tissue and contents at one timepoint (table 1&2, figure 1&2). It is possible this represents over-supplementation. In other reports, carbohydrate supplementation beyond what that used by the crop resident flora resulted in an increase in pathogen growth (31). Future studies will compare *Lactobacillus acidophilus* levels between control, challenge control, and treated and challenged pouls as it is not known if *L. acidophilus* levels were increased by providing it in the drinking water.

Supplementation affected crop emptying as well. Whole crop weight decreased (Table 5) throughout the first day and rebounded completely for the non-challenged pouls and partially for the other two groups. Pouls on the lactose and *L. acidophilus* supplement had lesser crop ingesta weights (than the non-challenged groups) on the second day. Challenged pouls also had slower crop emptying as compared to controls, though by the eight hour timepoint all groups had nearly empty crops. The pouls in group C had more *Salmonella* in
their crop tissue at eight and 24 hours according to culture results. This may indicate more illness and less appetite.

Another observation taken from this study was that crop contents taken from turkeys during the dark or night cycle were relatively dry, therefore it does not appear that birds were ingesting the supplement in the night before the challenge period. The effects of the lactose/Lactobacillus acidophilus addition should have been in place and protective against challenge as was seen in other studies.

*Salmonella typhimurium* was associated with feed and the crop epithelium as revealed by IHC. It appeared that the *Salmonella* propagated in the crop and then due to impaired gastric emptying, left the crop more slowly in group C poults than group B birds (indicated by culture results). The IHC did not show *Salmonella* invasion into the tissue in either challenged group. Bacteria were confined to the outer surface of the mucosa and appeared to slough off with the epithelium. This study did not take intestinal sections but it is possible that the slower crop clearance could lead to higher cecal contamination rates due to a longer inoculation period. The opposite could be true as well if the intestinal flora were protective against challenge. Future work could include a cecal comparison with crop colonization.

The feed in this study was a crumbled, commercially available diet. Other studies have used mash diets which could make a difference in fermentation of ingesta and pH of the crop environment and thus effectiveness of the lactose and *L. acidophilus* addition. Volatile fatty acid production has been shown to be an indicator of a favorable crop environment that is resistant to *Salmonella* colonization. Samples were collected for volatile fatty acid analysis but at this time have not been assayed due to potential difficulties in sample preparation.

Future work, including this analysis and comparison between feed types and fermentation
products may give a clearer picture of the influence of feed on Salmonella susceptibility in turkeys.

**Conclusion**

A model has been developed for *Salmonella typhimurium* colonization of the crop in the turkey. The approach used *Salmonella typhimurium* administration by oral gavage, quantification of *S. typhimurium* in the crop wall and ingesta and IHC of the crop wall section. The model can be employed to examine the effectiveness of various techniques to reduce Salmonella contamination in the turkey crop.

The application of a *Lactobacillus acidophilus* and lactose supplement in the drinking water of turkey poults until three weeks of age did not significantly reduce the recovery of *Salmonella typhimurium*. The supplementation used in this study instead seemed to aid in *S. typhimurium* propagation in the turkey crop.
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My fiancée Tim has been and continues to be unshakable support without whom the research reviewed here would not have been possible. I feel so fortunate to have you in my life. I would also like to thank my committee members for their guidance and patience through the years. I have learned so much and I hope to repay you in the years to come.

Finally, I would like to thank my Aunt Carol, the sole benefactor to the “Sara should finish her MS” scholarship program. You are my role model for professional determination.

“Go confidently in the direction of your dreams. Live the life you have imagined.”

Henry David Thoreau
Table 1 Changes in the *Salmonella typhimurium* (log 10) CFU recovered in crop ingesta with time and pretreatment\(^1\) in the drinking water. Three-week-old turkey poults challenged with *Salmonella typhimurium*\(^2\) received supplement from one day of age.

<table>
<thead>
<tr>
<th>Time (hours post challenge)</th>
<th>Control (B)</th>
<th>Lactose + <em>Lactobacillus acidophilus</em> Supplement (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>7.00 +/- 0.29 (5)(^{ax})</td>
<td>7.15 +/- 0.27 (6)(^{ax})</td>
</tr>
<tr>
<td>4</td>
<td>5.92 +/- 0.27 (6)(^{ax})</td>
<td>7.79 +/- 0.29 (5)(^{bx})</td>
</tr>
<tr>
<td>8</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>24</td>
<td>4.20 +/- 0.29 (5)(^{ay})</td>
<td>4.97 +/- 0.27 (6)(^{ay})</td>
</tr>
</tbody>
</table>

Analyzed by 2 way ANOVA (excluding non-challenge poults) with SAS (Carey, NC), excluding 8 hour timepoint which had <1 g crop content (NA). Treatment effect p=0.0009, time p=<0.00014, trt*time p=0.0169

Superscripts \(^{a,b}\) indicate difference with treatment p< 0.05 by Tukey-Kramer range test
Superscripts \(^{x,y}\) indicate difference with time p<0.05 by Tukey-Kramer range test p<0.05 for treatments and between times 0.5 to 24 hours and 4 to 24 hours

\(^1\)2.5% Lactose and *Lactobacillus acidophilus* 1x10\(^9\) per liter
\(^2\)1.7x10\(^8\) CFU/ml orally
Table 2 Changes in the *Salmonella typhimurium* (log 10) CFU recovered in crop wall tissue with time and pretreatment\(^1\) in the drinking water. Three-week-old turkey poult\(s\) challenged with *Salmonella typhimurium*\(^2\) received supplement from one day of age.

<table>
<thead>
<tr>
<th>Time (hours post challenge)</th>
<th>Control (B)</th>
<th>Lactose + <em>Lactobacillus acidophilus</em> supplement (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5.66 +/- 0.245 (6)(^x)</td>
<td>5.69 +/- 0.223 (6)(^x)</td>
</tr>
<tr>
<td>4</td>
<td>4.97 +/- 0.223 (6)(^x)</td>
<td>5.94 +/- 0.245 (5)(^x)</td>
</tr>
<tr>
<td>8</td>
<td>5.49 +/- 0.223 (6)(^x)</td>
<td>5.19 +/- 0.223 (6)(^x)</td>
</tr>
<tr>
<td>24</td>
<td>3.58 +/- 0.316 (3)(^y)</td>
<td>3.93 +/- 0.223 (6)(^y)</td>
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</table>

Analyzed by 2 way ANOVA (excluding non-challenge poult\(s\)) with SAS (Carey, NC)
Treatment effect p=0.5411, time p=.0001, trt*time p=0.0730

Superscripts \(^x\)\(^y\) indicate difference with time p<0.05 by Tukey-Kramer range test. There were no significant differences between treatments. Time differences between the 24 hr and 0.5,4,8 time points p<0.05

\(^1\)2.5% Lactose and *Lactobacillus acidophilus* 1x10\(^9\) per liter
\(^2\)1.7x10\(^8\) CFU/ml orally
Table 3 Changes in the crop ingesta weight with time and pretreatment\(^1\) in the drinking water. Three-week-old turkey poults challenged with *Salmonella typhimurium*\(^2\) received supplement from one day of age.

<table>
<thead>
<tr>
<th>Time (hours post challenge)</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No challenge (A)</td>
<td>Control (B)</td>
<td>Lactose + <em>Lactobacillus acidophilus</em> supplement (C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28.05 +/- 2.84 (6)(^\text{y})</td>
<td>23.19 +/- 3.11 (5)(^\text{x})</td>
<td>28.35 +/- 2.84 (6)(^\text{y})</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>11.47 +/- 2.84 (6)(^\text{x})</td>
<td>21.58 +/- 2.84 (6)(^\text{x})</td>
<td>18.61 +/- 3.11 (5)(^\text{xy})</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>25.9 +/- 2.84 (6)(^\text{by})</td>
<td>14.98 +/- 3.11(^\text{abx})</td>
<td>8.38 +/- 2.84 (6)(^\text{ax})</td>
<td></td>
</tr>
</tbody>
</table>

Analyzed by 2 way ANOVA with SAS (Carey, NC)
No 8 hour sample because crop content was <1 g (NA)
Treatment effect p=0.3633, time p= <0.0001, trt*time p=0.0004

Superscripts \(^a,b\) indicate difference with treatment p<0.05 by Tukey-Kramer range test. Superscripts \(^x,y\) indicate difference with time p<0.05 by Tukey-Kramer range test. There were no treatment effects. Time effects p<0.05 for 0.5 to 4 hours and 0.5 to 24 hours.

\(^1\)2.5% Lactose and *Lactobacillus acidophilus* 1x10\(^9\) per liter
\(^2\)1.7x10\(^8\) CFU/ml orally
Table 4  Effect of pretreatment (2.5% Lactose and *Lactobacillus acidophilus* 1x10^9) on crop and content weight in 3 week old turkey poults (log10) challenged with *Salmonella typhimurium* (1.7x10^8 CFU/ml orally)

<table>
<thead>
<tr>
<th>Time (hours post challenge)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No challenge (A)</td>
</tr>
<tr>
<td>0.5</td>
<td>35.24 +/- 2.56 (6)^y</td>
</tr>
<tr>
<td>4</td>
<td>17.88 +/- 2.56 (6)^xy</td>
</tr>
<tr>
<td>8</td>
<td>7.13 +/- 2.81 (5)^x</td>
</tr>
<tr>
<td>24</td>
<td>32.81 +/- 2.56 (6)^by</td>
</tr>
</tbody>
</table>

Analyzed by 2 way ANOVA with SAS (Carey, NC)
Treatment effect p=0.1937, time p<0.0001, trt*time p=0.0002

Superscripts a,b indicate differences with treatment p<0.05 by Tukey-Kramer range test.
Superscripts x,y indicate difference with time p<0.05 by Tukey-Kramer range test.
No significant differences between treatments. P<0.05 for all times except 4 to 24 hr.

1 2.5% Lactose and *Lactobacillus acidophilus* 1x10^9 per liter
2 1.7x10^8 CFU/ml orally
Table 5 Changes in the crop wall weight with time and pretreatment\(^1\) in the drinking water. Three-week-old turkey poults challenged with *Salmonella typhimurium*\(^2\) received supplement from one day of age.

<table>
<thead>
<tr>
<th>Time (hours post-challenge)</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No challenge (A)</td>
<td>Control (B)</td>
<td>Lactose and <em>Lactobacillus acidophilus</em> supplement (C)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>7.18 +/- 0.41 (6)</td>
<td>8.27 +/- 0.45 (5)</td>
<td>7.48 +/- 0.41 (6)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.42 +/- 0.41 (6)</td>
<td>7.31 +/- 0.41 (6)</td>
<td>6.44 +/- 0.45 (5)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.13 +/- 0.45 (5)</td>
<td>6.91 +/- 0.41 (6)</td>
<td>6.92 +/- 0.41 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Analyzed by 2 way ANOVA with SAS (Carey, NC)  
No 24 hour weight was taken  
Treatment effect p=0.2332, time p=0.0351, trt*time p=0.571  
No significant differences between rows or columns by Tukey-Kramer Range Test.

\(^1\)2.5% Lactose and *Lactobacillus acidophilus* 1x10\(^9\) per liter  
\(^2\)1.7x10\(^8\) CFU/ml orally
Figure 1. Changes in *Salmonella typhimurium* recovered from the crop ingesta of three week old turkey poults. Group C was receiving 2.5% lactose and $1 \times 10^9$ *Lactobacillus acidophilus* per liter in the drinking water.

![Bar chart showing changes in *Salmonella typhimurium*](image)

- Treatment B: challenge only
- Treatment C: supplement and challenge

Axes:
- x, y indicate difference over time p<0.05
- a, b indicate difference between treatment p<0.05

Time points:
- 1 = 0.5 hr, 2 = 4 hr, 3 = 8 hr
Figure 2. Changes in *Salmonella typhimurium* recovered from the crop wall of three week old turkey poults. Poults in group C were receiving 2.5% lactose and $1 \times 10^9$ *Lactobacillus acidophilus* supplement in the drinking water.

![Bar chart showing changes in *Salmonella typhimurium* levels.](image)

- Treatment B: challenge only
- Treatment C: challenge and supplement

Time points:
- 1 = 0.5 hr
- 2 = 4 hr
- 3 = 8 hr
- 4 = 24 hr

x, y indicate difference over time $p<0.05$
Figure 3. Changes in crop content weight of three week old turkey poultts challenged with *Salmonella typhimurium*. Group C received 2.5% lactose and $1 \times 10^9$ *Lactobacillus acidophilus* per liter in the drinking water.

A: negative control
B: challenge control
C: supplement and challenge

a,b indicate difference between treatments $p<0.05$

x,y indicate difference over time $p<0.05$
Figure 4. Changes in the whole crop weight (content and wall) of three week old turkey poults challenged with *Salmonella typhimurium*. Poults in group C received 2.5% lactose and $1 \times 10^9$ *Lactobacillus acidophilus* per liter in the drinking water.

A= negative control
B=challenge control
C=challenge and treatment

**Figure Details:**
- Time points: 1=0.5 hr, 2=4 hr, 3=8 hr, 4=24 hr
- a,b indicate difference between treatments $p<0.05$
- x,y indicate difference over time $p<0.05$
Figure 5. Changes in crop wall weights in three week old turkey poults challenged with *Salmonella typhimurium*. Poults in group C received 2.5% lactose and $1 \times 10^9$ *Lactobacillus acidophilus* in the drinking water.
Figure 6 A. Hematoxylin and Eosin staining of turkey crop section from time point two, four hours post challenge, group B that did not receive supplementation. 40X magnification.

Figure 6 B. Immunohistochemical staining against *Salmonella typhimurium* in the turkey crop. Same section as above (A): time point two four hours post challenge, group B that did not receive supplementation. 40X magnification
Figure 7. Immunohistochemical staining against *Salmonella typhimurium* in the turkey crop. Section is from time point one, 0.5 hours post challenge, group B that did not receive supplementation. 40X magnification

Figure 8. Immunohistochemical staining against *Salmonella typhimurium* in the turkey crop. Section is from time point one, 0.5 hours post challenge, group C which did receive supplementation. 100X magnification
Figure 9. Immunohistochemical staining against *Salmonella typhimurium* in the turkey crop. Section is from time point three, eight hours post challenge, group B that did not receive supplementation. 40X magnification

Figure 10. Immunohistochemical staining against *Salmonella typhimurium* in the turkey crop. Section is from time point three, eight hours post challenge, group C did receive supplementation. 100X magnification
Figure 11. Immunohistochemical staining against *Salmonella typhimurium* in the turkey crop. Section is from time point four, 24 hours post challenge, group B did not receive supplementation. 100X magnification.

Figure 12. Immunohistochemical staining against *Salmonella typhimurium* in the turkey crop. Section is from time point four, 24 hours post challenge, group C did receive supplementation. 40X magnification.