SURVIVAL OF SALMONELLA IN ORGANIC AND CONVENTIONAL BROILER FEED AT DIFFERENT TEMPERATURES AND WATER ACTIVITIES

by

APARNA PETKAR

(Under the Direction of Walid Alali)

ABSTRACT

The objective of this project was to compare the survival of Salmonella in organic versus conventional broiler feed stored at different temperatures (11, 25, and 38°C) and water activities (0.75, 0.55, and 0.43) over 80 days. Feed samples were inoculated with five Salmonella serotypes at high: $10^6$ and low: $10^3$ CFU/g doses. Salmonella populations (CFU/g) in the feed samples were analyzed quantitatively using direct plating (CFU/g) and qualitatively (enrichment when direct plating was negative). Although Salmonella populations were statistically significantly lower in organic feed for majority of temperature-by-aw over 80 days compared to conventional feed, differences in mean Salmonella populations were less than one log. Odds-ratio (OR) for Salmonella presence in conventional feed was significantly (P<0.05) higher than in organic, for high and low inoculum (OR = 4.76 and 2.92, respectively). Based on these findings, there were no biologically significant reductions in Salmonella populations in organic feed compared to conventional.

INDEX WORDS: Salmonella, Conventional poultry feed, Organic poultry feed, Temperature, Water activity
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APARNA PETKAR

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by

APARNA PETKAR

Major Professor: Walid Alali
Committee: Larry Beuchat
           Mark Harrison

Electronic Version Approved:

Maureen Grasso
Dean of Graduate School
The University of Georgia
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DEDICATION

I would like to dedicate this thesis to my parents whose endless love, constant support and encouragement has helped me throughout my life. I thank them for giving me this opportunity to prove myself and cannot express my gratitude towards them. They are my pillars. Last but not the least; I would also like to thank my husband Bhabesh for his unconditional love, support and inspiration. He always kept my spirits high during the graduate study and showed me a path in dark phases. I feel that this is as much the accomplishment of my family as its mine.
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CHAPTER 1

LITERATURE REVIEW

*Salmonella* is a Gram negative bacterium often associated with many foodborne diseases. This pathogen belongs to the family Enterobacteriaceae. *Salmonella* is rod shaped motile, non-spore-forming bacilli that ferments glucose, maltose, and mannitol sugars (Blackman et al., 1992). Most of the *Salmonella* spp. produce acid and gas due to fermentation of sugars (Franco, 1997). They can be serogrouped based on their somatic (O) antigens (composed of lipopolysaccharides) and flagellar (H) antigens (composed of flagellin protein). The genus *Salmonella* consists of more than 2,300 serovars. These organisms are not very fastidious and therefore can multiply over a wide range of conditions (Franco, 1997). They are not highly resistant to physical or chemical agents and can be killed by heating at 55°C for 1 hour, or by subjecting them to 60°C for 15 to 20 minutes (Franco, 1997). The optimal temperature required for their growth is 37°C, which is the normal human body temperature. *Salmonella* infections (i.e., salmonellosis) are zoonotic and can be transmitted from animals to humans and vice versa. It can also be transmitted via food to humans (i.e., foodborne illness). *Salmonella* is readily killed by standard cooking temperatures (70 to 80°C), pasteurization (75°C), and various disinfectants (e.g., tar oil phenol and quaternary ammonium biocide). *Salmonella* can survive freezing (-20°C) and refrigeration (4°C) temperatures. Additionally, this organism can survive desiccation and under optimum conditions, it can divide every 20 minutes (Franco, 1997).

Animal feed and *Salmonella* contamination have long history which can be dated back to the time when animals where first domesticated (Ellis, 1968). First reports of *Salmonella*
presence in poultry feed were made by Edwards et al. (1948). The authors reported the isolation of both \textit{S. Typhimurium} and \textit{S. Bareilly} from chicken feed. It was thought that source of these organisms was the egg powder in the feed. Wilson (1948) in Great Britain was among the first who suggested that \textit{Salmonella} other than \textit{S. Pullorum} and \textit{S. Gallinarum} can be transmitted to chicks from feed that have been contaminated with infected rodent feces. In 1952, Muller isolated and identified seven \textit{Salmonella} serotypes from 13 positives out of 329 meat and bone meal (MBM) samples that were imported into Denmark from South America and Australia. They also identified contaminated feed as a potential source for introduction and spread of salmonellosis among domestic animals. Erwin (1955) first reported the isolation of \textit{Salmonella} from commercially prepared and distributed poultry feed in United States. From a total of 206 samples including mash, pellets, concentrates, and granulated feed, they identified three isolates of \textit{S. Oranienburg}. At the same time, scientists (1955) detected \textit{Salmonella} in 5 of 40 (12.5\%) samples from imported fish meal that was supposed to be used in poultry feed manufacture. Researcher (1956) examined 275 imported fish and meat meal samples for the presence of \textit{Salmonella} spp. Authors reported that 15.6\% of samples contained \textit{Salmonella}, including 22 different serotypes. Hence, the authors recommended that imported animal feed should be bacteriologically screened for \textit{Salmonella} before selling to poultry growers. Seeliger (1956) reported the serotype distribution, frequency, and method of spread of \textit{Salmonella} infection in animals. In the same paper, they also mentioned different methods for prevention of \textit{Salmonella} infection in animal feed.

Muller (1957) attributed the occurrence of \textit{Salmonella} infections in poultry to importation of large quantities of contaminated MBM, blood meal, bone meal, and fish meal. Boring (1958) tested 16 samples of fish meal from eight areas along the Atlantic and Pacific coast. Nine of the
16 were found positive for *Salmonella* and 11 serotypes were identified. Due to the large amount of fish meal used in the poultry feeds, they also discussed the dangers of *Salmonella* contamination in poultry feed. Boyer *et al.* (1958) suggested a direct relationship between *Salmonella* infections in turkey poultts and presence of the organism in the poultry feed. Six *Salmonella* serotypes were identified in 5 unopened bags of feed out of 33 commercial starter mash bags sampled. He rendered the possible cause of this phenomenon to the poor handling and storage of commercial feed.

In 1958, an outbreak of human salmonellosis caused by rare serotype *S*. Hadar was investigated by Hirsch and Sapiro-Hirsch in Israel. It was suggested that chicken livers (contaminated with *Salmonella*) were the source of infection. Interestingly, poultry feed that contained poultry by-products and bone meal fed to the chickens were also found to be contaminated with the same serotype that was isolated from salmonellosis patients.

According to review by Williams (1981b) on *Salmonella* in feed, a scientist named Bergsma in Netherland suggested that imported poultry feed ingredients were often contaminated with *Salmonella*. They suggested that poultry is one of the main reservoirs of *Salmonella* due to the use of contaminated feed ingredients. Kovacs (1959) found that meat meals especially from Australia were heavily contaminated. Hence, he recommended that meat meal should be heat treated so as to eliminate *Salmonella*.

Later, *S*. Binza was isolated from two fish meal samples in Angola (Williams, 1981b) and observed that the isolated *Salmonella* strain was pathogenic to the chicks. When the chicken feed, infected with *Salmonella* containing fish meal was fed to the chicks, it promoted the production of antibodies in a large percentage of the birds. Taylor (1960) in England reported to
have found 72 *Salmonella* serotypes in animal feeds. According to her, the reduction in *Salmonella* infection would have tremendous effect on general incidence of *Salmonella* infection in poultry feed.

Carlson and Snoeyenbos (1970) demonstrated that condensation in ingredient storage containers may provide microenvironment capable of supporting growth of *Salmonella* spp. Kumar *et al.* (1971) reported that *Salmonella* could be transmitted to the turkey flock via contaminated feed. They also isolated *S*. Kentucky from feed samples and cloacal swabs of poults that were fed on this contaminated feed. In 1974, MacKenzie demonstrated association between high mortality in *Salmonella* infected broiler flocks and increased incidence of *Salmonella* contamination of feed grain constituents by tracing the organisms from grain to breeder feed, carrier birds, and ultimately to dressed broilers. Lee (1974) recognized that contaminated animal feed plays a significant role in maintenance of *Salmonella* reservoirs in poultry flocks. Moreover, Watson and Brown (1975) suggested a direct relationship between *Salmonella* in poultry feed and poultry birds. They also suggested that salmonellosis can be best prevented by the use of clean feed. In 1978, the U.S. Advisory Committee on *Salmonella* (Anon., 1978b) admitted that feed is the most important source of infection in poultry and livestock and research on this subject should be addressed with utmost importance.

It is well documented that the incidence and the frequency of *Salmonella* is influenced by several factors such as antibacterial factor, water activity, and temperature. In order to survive in feed, *Salmonella* must combat the same environmental conditions as nonpathogenic microflora. In 1978, William and Benson found that both primary antibacterial factor inherent in feed and low water activity were not completely effective in destroying *Salmonella* populations in feed as *Salmonella* spp. were isolated from the poultry feed stored at 25°C for 16 months stored. In
1984, Juven et al. demonstrated that the survival of *Salmonella* was greater at water activity of 0.43 than at 0.75. Several investigators observed the survival and heat resistance of *Salmonella* spp. in meat and bone meal, dry milk, and poultry feed to be inversely proportional to moisture content and relative humidity, except at the moisture level that allows growth (Liu et al., 1969; Carlson and Snoeyenbos, 1970; Juven et al., 1984). To the best of our knowledge, there were no reports that examined the survival of *Salmonella* under various combinations of temperatures and water activities, and in different types of feed.

In summary, foods of animal origin including poultry meats can serve as an important source of foodborne salmonellosis. The transmission of *Salmonella* in the environment has been predicted as cyclic. This means that disease cycle can be continued again and again in the presence of various infection sources (dust, water, feed, and chicken feces) in the poultry house. Moreover, contaminated poultry feed have been suggested as an important source of poultry infection with *Salmonella* (Williams, 1981 a, b). Interestingly, feed can serve as both direct and indirect source of transmission. However, depending upon whether the individual feed ingredients were originally contaminated before or during feed mixing or whether the mixed feed gets contaminated during feeding, feed can serve as both direct and/or indirect route of *Salmonella* transmission to the poultry. There is very little information available regarding the relative significance of different sources of contamination but eventually the birds are exposed to foodborne *Salmonella* during consumption of contaminated feed. Therefore, identification of potential sources of feed contamination and the factors that influence the survival of *Salmonella* in feed, are of high significance to the poultry industry to limit *Salmonella* contamination and presence in poultry feed.
LITERATURE CITED


CHAPTER 2

SURVIVAL OF *SALMONELLA* IN ORGANIC AND CONVENTIONAL BROILER FEED AT DIFFERENT TEMPERATURES AND WATER ACTIVITIES

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ABSTRACT

Salmonellosis is one of the leading causes of foodborne disease in the United States and around the world. In the poultry industry, transmission of Salmonella to the broiler birds has been associated with contaminated poultry feed. Contamination of feed with Salmonella can occur during feed processing and storage. Current work in our laboratory showed that the prevalence of Salmonella was higher in conventional broiler feed than in organic feed. Previous studies have examined the effect of either temperature or water activity (a_w) on survival of Salmonella in conventional poultry feed. However, there is very limited information published on the effect of both a_w and temperature on the survivability of Salmonella in certified-organic broiler feed versus conventional feed. The objective of this experimental study was to compare the survival of Salmonella in organic versus conventional broiler feed stored at different temperatures and water activities over an 80-day period. Two Salmonella inocula (high: $10^6$ and low: $10^3$ CFU/g of feed) were used in the study. Five Salmonella enterica serotypes were used in the feed inoculation. The effect of temperature and a_w on the feed samples (organic and conventional) at both inocula was assessed using a 3x3 factorial experimental design. Three temperatures (11, 25, and 38°C) and three a_w (0.75, 0.55, and 0.43) were used to simulate the different possible feed storage conditions at conventional and organic poultry production environment. The concentrations (CFU/g) of Salmonella in the feed samples were measured at days 0, 3, 7, 14, 21, 28, 35, 50, 65, and 80. At each sampling point, feed samples (in triplicates) were analyzed for Salmonella counts using direct plating. Enrichment technique was used when direct plating resulted negative. Although Salmonella populations (mean CFU/g) were statistically significantly lower in organic feed at the both high and low doses and for the majority of temperature-by-a_w over the 80 days of storage compared to conventional feed, the
differences in mean *Salmonella* populations between both feed types were less than one log$_{10}$. The odds-ratio (OR) for *Salmonella* presence in conventional feed was significantly (P<0.05) higher than in organic feed, for high and low inoculum (OR = 4.76 and 2.92, respectively). Based on these findings, there were no biologically significant reductions in *Salmonella* populations observed at both inoculum levels in organic broiler feed compared to conventional feed, while significant reduction was observed in proportion of *Salmonella* positives in organic feed compared to conventional feed at both inocula.

**INTRODUCTION**

*Salmonella* is one of the most common pathogens to cause foodborne disease in the United States and worldwide. According to Mead et al. (1999), nearly 1.4 million people suffer from salmonellosis annually in the U.S. Poultry and poultry products are considered to be an important source of *Salmonella*-related foodborne disease in humans (Tauxe, 1991; Bryan and Doyle, 1995). The presence of *Salmonella* in the production environment and in or on the bird is a major concern for poultry industry and contaminated feed is considered one of the main sources of *Salmonella* infection in broiler birds (Jones *et al*., 1991; Maciorowski *et al*., 2004). The presence of *Salmonella* in poultry feed has been documented in both poultry feed and feed ingredients (Williams 1981a, Cox *et al*., 1983, Stuart 1984, Veldman *et al*., 1995 Ha *et al*., 1997, 1998). Moreover, *Salmonella* contaminated poultry feed have been associated with feed matrices like grain, oilseed meal, feather and fishmeal, meat by-products. As contaminated feed is consumed by birds, *Salmonella* can multiply in the gastrointestinal tract of the bird, and could eventually be shed in high populations in the feces through grow out (Cason *et al*. 1994). Several interventions methods including heating and pelleting at 70°C and 90°C, irradiation (gamma rays), chemicals (organic acids and their salts, formaldehyde, and bacterial membrane disruptors
such as terpenes and essential oils) have been proposed to control *Salmonella* populations in poultry feed and feed ingredients (Wilder, 1969; Hinton and Linton, 1988). Despite this, poultry feed can be recontaminated with *Salmonella* during storage at farm level (Hinton and Linton, 1988). The storage of poultry feed under various environmental conditions (i.e., temperature and humidity) may influence the survival of *Salmonella* which could subsequently impact the load of this organism present in feed and consumed by broiler birds. This information would be helpful to identify improper storage conditions that favor *Salmonella* survival in contaminated poultry feed. Reports from several studies showed that the survival of *Salmonella* was influenced by several factors such as antibacterial factor, moisture, and temperature (Himathongkham et al., 1996, Halls and Tallentire 1978, Furuta et al., 1980a, McCapes et al., 1989). Juven et al. (1984) demonstrated that the survival of *Salmonella* was greater at water activity of 0.43 than at 0.75. Several investigators observed the survival and heat resistance of *Salmonella* spp. in meat and bone meal, and poultry feed to be inversely proportional to moisture content and relative humidity (Relative humidity=$a_w \times 100\%$), except at the moisture level that allows growth (Liu et al., 1969; Carlson and Snoeyenbos, 1970; Juven et al., 1984). In a recent study by Alali et al. (2010), authors showed that conventional feed was contaminated with *Salmonella*, whereas USDA-certified organic feed was *Salmonella*-free. It is unclear whether organic feed was contaminated with *Salmonella* at storage, but the organism did not survive to a culturable level. Therefore, the objective of this study was to compare the survival of *Salmonella* in USDA-certified organic versus conventional broiler feed stored at different temperatures and $a_w$ over 80-day period.
MATERIALS AND METHODS

Salmonella Serotypes:

Five *Salmonella enterica* serotypes were obtained from the Poultry Diagnostic Research Center, University of Georgia. These serotypes were: *S*. Typhimurium, *S*. Heidelberg, *S*. Enteritidis, *S*. Montevideo, and *S*. Gaminara. These serotypes were propagated in tryptic soy broth (TSB) for 24 h at 37°C incubation. Then, a loopful of TSB broth for each serotype was streaked on a thin agar layer tryptic soy agar (TSA)-xylose lysine tergitol (XLT4) agar incubated at 37°C for 24 h, to obtain pure colonies. The TSA-XLT4 agar was prepared in our laboratory as described in (Kang and Fung, 2000). This agar is generally used for direct plating to enumerate and recover injured *Salmonella*. It was composed of a bottom layer of XLT4 selective medium and top thin layer of TSA nonselective agar.

**Salmonella inoculum:**

To prepare the inoculum, 6 ml of nutrient broth (NB) was inoculated with a single colony of each *Salmonella* serotype from a 24 h TSA-XLT4 culture. The NB inoculum concentration was adjusted to approximately $10^8$ colony forming units (CFU)/ml using a spectrophotometer (Spectronic 20; Bausch and Lomb, Rochester, NY) (optical density at 600 nm = 0.5-0.6, ≈$10^8$ CFU/ml) as described in Kaiser *et al.* (2002). This inoculum concentration ($10^8$ CFU/ml) was used to prepare a high dose dry chalk inoculum. Ten-fold serial dilutions were made to generate low dose inoculum ($10^5$ CFU/ml) and were used to prepare a low dose dry chalk inoculum.
Dry chalk inoculum:

A dry-chalk Salmonella inoculum method was adopted from Okelo et al. (2008) to use in our study. Blocks of chalk used for in this study were obtained from Staples, Inc. (McDonough, GA). Prior to use, the blocks were autoclaved and made sterile. The pH of the chalk was neutral, approximately 7.0 measured by a pH meter (Fisher Scientific, Pittsburgh, PA). Blocks of chalk (4.33 g) were weighed and aseptically submerged in a cocktail of five Salmonella culture NB (6 ml) broth for 12 h. The NB contained approximately $10^8$ and $10^5$ CFU/ml so as to obtain high and low dose inocula, respectively. Inoculated chalks were then placed on sterile petri dishes and placed in a 37°C incubator for drying. These chalks were allowed to dry for approximately 72 h back to their original weight (4.33g). The blocks of chalk were then pulverized using grinder in a laminar air flow chamber to obtain a powdered inoculum of approximately $10^7$ and $10^4$ CFU/g of chalk. After drying, $a_w$ of chalk powder was 0.22.

Enumeration of Salmonella in dry chalk inoculum:

Enumeration of viable Salmonella cells was performed by adding 10 g of high and low chalk inocula to 90 ml of phosphate buffered saline (PBS) to make 100 ml of suspension. Subsequent 10-fold serial dilutions were made in PBS and 100 μl aliquots of dilutions ($10^{-1}$ to $10^{-5}$) were plated in duplicate on TSA-XLT4 plates and incubated at 37°C for 24 h.

Feed samples:

Conventional pelleted feed (formulated for grower birds) (Table 4) was purchased from two different conventional poultry companies (company A and B). The listed feed formulation was similar in the two companies. The pH of the two conventional poultry feed were 5.80 and 5.90. Organic mash feed (formulated for grower birds) (Table 3) was obtained from two different
organic poultry companies (company C and D) with similar listed formulation. The pH of two organic composite feed were 5.62 and 5.70. The $a_w$ values for conventional and organic feed was 0.30 and 0.29, respectively. Prior to feed inoculation, feed samples were tested to ensure that they were negative for *Salmonella*.

**Preparation of feed samples with desired water activities:**

Secador desiccator cabinets (Structure Probe, Inc., West Chester, PA) were used to store the feed and to reach the desired relative humidities prior to inoculation. Nine desiccators were kept at three different temperatures (11, 25 and 38°C). Different amounts of saturated salt solution (potassium carbonate, sodium bromide and sodium chloride) were placed inside each desiccator to attain the desired relative humidities as recommended by Rockland (1960). The desired water activities for our project were 0.43, 0.55 and 0.75. For a 3-week period, feed samples (prior to inoculation) were placed inside the desiccators with the salt solutions to attain the desired $a_w$. Water activity in feed was measured over time using Aqualab water activity meter (Decagon devices, Inc. Pullman, WA).

**Study design:**

The whole experiment was replicated twice. We used feed from company A and C for the first trial; whereas feed from company B and D was used in the second trial. A 3 x 3 factorial design was used to conduct the study. For each of the two inocula, three different temperatures (11, 25, and 38°C) and three different water activity levels (0.75, 0.55 and 0.43) were used to determine the survival of *Salmonella* in organic and conventional broiler feed over 80-day period. For each temperature $\times a_w$ combination, 9 g portions of conventional and organic feed, in triplicate, were inoculated with 1 g of the dry-powder chalk containing *Salmonella* to obtain final concentrations of $10^6$ (high) and $10^3$ (low) CFU/g. The samples were stored in 15 ml sterile glass
sealed tubes, mixed homogenously by vortexing for 1-2 min, and then placed back inside the desiccator

**Salmonella analysis from feed samples:**

A 10 g portion from every temperature-a$_w$ combination, in triplicate, was taken at each sampling date over the 80 days for *Salmonella* analysis. The sampling days were: 0, 3, 7, 14, 21, 28, 35, 50, 65, and 80. For the high inoculum samples, the 10 g samples were suspended in a 90 ml of LB in a sterile flask, followed by shaking for an hour in a rotary shaker (New Brunswick Scientific, Edison, NJ). Later, 10-fold serial dilutions were made from the suspension in a sterile micro-centrifuge tubes containing 1 ml PBS. One hundred microliters aliquots from each dilution ($10^1$ to $10^3$) were spread plated on TSA-XLT4 plates. After 24 h of incubation at 37°C, bacterial colonies were enumerated. For the low inoculum samples, *Salmonella* enumeration was done as above with the exception of no serial dilutions was performed. Feed samples suspended in LB were directly spread plated on TSA-XLT4 medium.

**Selective enrichment:**

Enrichment was performed when direct plating was negative after period of incubation of 24 h. Briefly, 1 ml and 0.1 ml of aliquots of feed suspended- LB were transferred into 10 ml of tetrathionate broth (TT) broth and 10 ml of rappaport vassiliadis broth (RV) broth, respectively. After, 24 h of incubation at 42°C (RV broth) and 35°C (TT broth), a loopful of the culture was streaked on TSA-XLT4 medium. Finally, after a period of incubation for 24 h at 37°C, presence or absence of *Salmonella* was determined by presumptive colony characteristics and biochemical test on triple sugar iron agar slants (TSI). The tubes were incubated with caps loosen at 35°C.
and examined after 18-24 h for carbohydrate fermentation, gas production and hydrogen sulfide production.

**DATA ANALYSIS**

The experimental trial had two outcomes of interest. Firstly, the population of *Salmonella* (log$_{10}$ CFU/g) by direct plating was compared among each temperature × a$_w$ combination, by inoculum dose level, and at each sampling time-by-a$_w$ combination using repeated measures analysis of variance ANOVA in General Linear Model in SAS software version 9.1.3 (GLM procedure, SAS Inst., Inc., Cary, NC). *Salmonella* counts were logarithmically transformed by use of log base 10 to approximate normality. Data from the two replicate experiments were tested using Levene’s test for homogeneity of variances. The variances between the two replicate experiments did not differ ($P < 0.05$). Therefore, data from both replicates were pooled to obtain a set of 6 observations for each sampling point. Secondly, the proportion of samples positive for *Salmonella*. A sample was considered positive if either direct plating was positive or enrichment was positive, when direct plating was negative. The proportion of positive samples for *Salmonella* was compared among each temperature-by-a$_w$ combination, by inoculum dose level, and at each sampling time using a GLM with a binomial distribution and a logit link in SAS (GENMOD procedure).

**RESULTS**

*Salmonella* populations were measured over 80 days period in inoculated conventional and organic broiler feed at different temperature and a$_w$. All feed samples were negative for *Salmonella* prior to inoculation. Over all temperatures and a$_w$, there were statistically significant differences ($P < 0.05$) in *Salmonella* populations between feed type (conventional and organic) and time (days) for both inoculum levels. For the high inoculum, the means log$_{10}$ CFU of
*Salmonella* per gram of feed ± SE were 4.71 ± 0.09 and 4.36 ± 0.09 for conventional and organic feed, respectively. For the low dose, the means log₁₀ of *Salmonella* were 2.88 ± 0.08 and 2.38 ± 0.08 for conventional and organic feed, respectively. There were no significant interaction effects (feed type x time) (P >0.05) observed for both inoculum level.

At day 0, the mean *Salmonella* populations in organic and conventional feed for high and low inoculum level was ~10⁶ and 10³ CFU/g, respectively. The effect of the different temperature and *a*ₜₐᵣᵢₜ combinations on *Salmonella* populations, by feed type and inoculum level over 80 day period, is shown in Tables 2-1 and 2-2. The mean *Salmonella* population in organic and conventional feed for both inoculum and each temperature-by-*a*ₜₐᵣᵢₜ combination over the study period is shown in Figures (2-1) – (2-18). For the high inoculum, there were statistically significant differences (P<0.05) at the storage temperature 11°C under the three different *a*ₜₐᵣᵢₜ; whereas no significant differences were observed at storage temperature 38°C (Table 2-1). The mean *Salmonella* populations in organic feed were lower than conventional feed for all temperature-by-*a*ₜₐᵣᵢₜ combinations (Table 2-1). Similarly, at conditions of 25°C x 0.55 and 25°C x 0.75 *Salmonella* populations were statistically significantly different, except for 25°C x 0.43. Although, 11°C x 0.75 and 25°C x 0.55 showed statistically significant differences between the two feed types, however; the difference in the mean was too small (<1.0 log) to be considered biologically meaningful. For the low inoculum, there were significant differences (P<0.05) at all storage temperature and *a*ₜₐᵣᵢₜ combinations. The mean *Salmonella* populations in organic feed were lower than conventional feed for all temperature-by-*a*ₜₐᵣᵢₜ combinations (Table 2-2). Although, the mean *Salmonella* populations showed statistically significant differences between the conventional and organic feed types for following temperature-by-*a*ₜₐᵣᵢₜ combination: 11°C x 0.43,
11°C x 0.55, 25°C x 0.55, 38°C x 0.55, and 38°C x 0.75; however, the differences in mean populations was too small (<1.0 log) to be considered biologically meaningful.

Over all temperature and a_w, the odds-ratio (OR) for _Salmonella_ presence in conventional feed was significantly (P<0.05) higher than in organic feed, for the high inoculum (OR = 4.76 [95% confidence interval {CI}, 2.66 to 22.57). For the low inoculum, the OR for _Salmonella_ presence in conventional feed was significantly (P<0.05) higher than in organic feed OR = 2.92 [95% CI, 2.16 to 4.53]. The GLM model comparing _Salmonella_ populations in conventional feed versus organic feed (as the referent category) failed to converge due to the sparse data (no or few positives) in most temperature×a_w combination at both inoculum levels. There were no significant differences in _Salmonella_ populations in conventional feed versus organic feed in the remaining temperature×a_w combination (data not shown).

In conventional feed (high inoculum dose), mean _Salmonella_ populations (log_{10} CFU/g) after 80-days period in stored feed at conditions of 11°C x 0.43, 25°C x 0.43, and 38°C x 0.43 were 6.16, 5.29, and 2.69, respectively (Table 2-1). At similar conditions in organic feed (high inoculum dose), mean _Salmonella_ populations (log_{10} CFU/g) after 80-days period in stored feed were 5.93, 5.05, and 2.52, respectively (Table 2-1). When conventional feed (high inoculum dose) were stored at conditions (11°C x 0.55, 25°C x 0.55, and 38°C x 0.55), mean _Salmonella_ populations (log_{10} CFU/g) recovered after 80-days of storage were 6.21, 5.55, and 2.86, respectively (Table 2-1). At similar storage conditions, _Salmonella_ populations (log_{10} CFU/g) from the organic feed recovered were 5.93, 5.09, and 2.44 respectively. Mean _Salmonella_ populations (log_{10} CFU/g) after 80-days of storage in conventional feed at conditions of 11°C x 0.75, 25°C x 0.75, and 38°C x 0.75 were 6.31, 5.67, and 2.44, respectively. In organic feed,
stored at similar conditions, mean *Salmonella* population recovered after 80-days of storage were 6.16, 4.79, and 2.22, respectively (Table 2-1).

In conventional feed (low inoculum) stored at conditions of 11°C x 0.43, 25°C x 0.43, and 38°C x 0.43, *Salmonella* survived at mean population (log₁₀CFU/g) of 4.29, 3.26, and 1.10, respectively (Table 2-2). At similar conditions in organic feed, mean *Salmonella* populations (log₁₀CFU/g) survived to the level of 4.12, 2.64, and 0.80, respectively (Table 2-2). When conventional feed were stored at conditions of 11°C x 0.55, 25°C x 0.55, and 38°C x 0.55, mean *Salmonella* populations recovered after period of storage were 4.58, 3.89, and 1.39, respectively (Table 2-2). Similarly, at above stated conditions, mean *Salmonella* populations in organic feed were 4.24, 3.15, and 1.06, respectively. At storage conditions of 11°C x 0.75, 25°C x 0.75, and 38°C x 0.75, mean *Salmonella* populations (log₁₀CFU/g) in conventional feed after period of 80-days were 4.43, 2.96, and 0.93, respectively. In organic feed, mean *Salmonella* populations (log₁₀CFU/g) survived after storage period of 80 days at similar storage conditions were 4.08, 1.86, and 0.81, respectively (Table 2-2).

**DISCUSSION**

To the best of our knowledge, this is the first study that compared the survival of *Salmonella* in organic versus conventional broiler feed under three temperatures and three aw. Over the past decades, poultry feed has been considered an important vehicle for the transmission of *Salmonella* to broiler birds since *Salmonella* spp. have been detected in animal feed, poultry feed and feed ingredients (Williams, 1981a; Cox et al., 1983; Stuart, 1984; Veldman et al., 1995). In our experiments, we used a dry inoculation technique of *Salmonella* (adapted from Okelo et al., 2008) into the feed rather than a culture-suspension inoculation. The
advantages of using dry *Salmonella* inoculum are: 1) it does not alter the $a_w$ of the feed. Hence, changes in feed background microbial flora is expected to be minimum (Hoffmans and Fung, 1993), 2) dry inoculum mimic *Salmonella* contamination of broiler feed on farm (including storage in feed silos) and during production/storage at the feed mills, and 3) because of low water activity, mold growth is expected to be minimal.

In this study, we included three different temperatures and $a_w$ combinations to mimic different possible feed storage conditions at the feed mills and on-farm during the different seasons. Since, dry poultry feed are usually marketed at $a_w$ ranging from 0.45 to 0.75 combined with different average stored feed temperatures experienced during winter and fall (11°C), spring (25°C), and summer (38°C) in Southeast U.S. Hence, attempts were made to include the range of possible environmental conditions for poultry feed storage along the production-to-consumption chain. In order to mimic different possible contamination levels, poultry feed were inoculated with high and low doses of *Salmonella* populations and evaluated over the storage period. Since poultry feed (conventional and organic) are not generally stored for more than 2 to 2.5 months at the farm and feed mill, the period of 80-days were chosen to evaluate the survivalability of *Salmonella* in feed. Due to the large sample size used in this study (n=180 observation for each temperature-by-$a_w$ combination); the statistically significance of *Salmonella* populations in organic feed versus conventional feed at most temperature-by-$a_w$ combinations at both inoculum levels would not be considered biologically meaningful. The differences in the mean *Salmonella* populations were too small (<1.0 log). The difference in organic and conventional feed composition shown in Table 2-3 and 2-4 did not appear to be a factor impacting the survival of *Salmonella* populations (counts); however, it may impacted the percentage of organic feed samples positive for *Salmonella* compared to conventional feed samples overall temperatures
and $a_w$. A sample was considered positive if either direct plating was positive or enrichment was positive, when direct plating was negative. A possible explanation for this difference in survivability of *Salmonella* in the two feed types is due to the absence of animal protein meals (blood and bone) and presence of natural antimicrobials in organic feed. Leuschner and Zamparini (2002) tested the effect of different natural antimicrobials such as garlic, ginger, mustard, and cloves on growth and survival of *E. coli* 0157 and *S. enterica* serovars Enteritidis in broth model systems. Garlic and clove showed bacteriostatic and bacteriocidal effect on both *E. coli* 0157 and *S. enterica* serovars Enteritidis, while clove was found to be more effective than garlic. Additionally, mustard and ginger also showed bacteriostatic activities against both bacteria. This may partly explain the reason for comparative survivability of *Salmonella* in organic than in conventional feed. While natural ingredients like ginger, garlic, and cloves are among the listed ingredients in organic feed that are lacking in conventional feed. The absence of these antimicrobial-factors might have contributed to better survival of *Salmonella* in conventional feed than in organic feed during the 80-days storage period.

Previous studies suggest that low moisture products such as peanut butter, infant formula, cereals, and dry aniseed (inherently low $a_w$ foods) do not support the growth of *Salmonella* (GMA, 2009). However, these products have been associated with outbreak of salmonellosis. Further, investigations suggest that factors such as poor sanitation practices, poor equipment design, improper maintenance, and poor ingredient control were responsible for outbreaks associated with these products (GMA, 2009). Also, *Salmonella* is known to survive for prolong periods of storage in low moisture products and thus making it difficult to control. During storage of these products, reduction in *Salmonella* population was observed however; this reduction depends on factors like storage temperature and product formulation. It was shown
that feeds with different ingredients but with similar moisture contents may influence \( a_w \) values, leading to different *Salmonella* survival rates (Duncan and Adams, 1972). In this study, *Salmonella* survived significantly less at both 11 and 25°C in organic versus conventional feed for both inoculum levels. Our study showed that at 11°C and \( a_w \) of 0.43, 0.55, and 0.75, *Salmonella* populations significantly declined in organic feed as compared to conventional feed over a storage period of 80 days. At storage conditions of 25°C and \( a_w \) of 0.55 and 0.75, *Salmonella* populations significantly declined in organic feed as compared to conventional feed. However, no significant effect were observed at \( a_w = 0.43 \) and 25°C between both feed types. Interestingly, 38°C and \( a_w \) of 0.43, 0.55, and 0.75, declines in *Salmonella* populations were not significantly different between conventional and organic poultry feed. Similar observations in conventional poultry feed were reported by (Williams and Benson, 1978), where *S. Typhimurium* survived much longer at 11 and 25°C than at 38°C. The study showed that in feed with high inoculum dose, the mean *Salmonella* populations recovered from both the feed types ranged from \( 10^4 - 10^5 \) CFU/g after 80 days of storage at conditions (11 and 25°C and three tested \( a_w \)). This observation was in concurrence with the findings of Davies and Wray (1996) where *S. Typhimurium* population declined to \(< 1.1 \times 10^3 \) CFU/g of conventional feed over a period of 3-months. The authors inoculated feed using a *Salmonella* suspension culture that led to osmotic shock thus lowering the level of viable *Salmonella* populations in wet inoculated feed as compared to the dry inoculated feed. According to several reports, inoculation procedure and \( a_w \) present in the feed may affect the survivability of *Salmonella* in feed samples (Liu et al., 1969a; Carlson and Snoeyenbos, 1970; Juven et al., 1984).

Several researchers showed that survival of *Salmonella* in dried products (e.g., fish meals, dry milk, poultry feed, cocoa powder, meat and bone meal) was influenced by \( a_w \) of the food and
feed product (Doesburg et al., 1970 and Juven et al., 1984). Interactions between a_w and environmental factors such as temperature may play an important role in the survival of Salmonella (Banwart and Ayres, 1956; Corry, 1976; Doesburg et al., 1970). Juven et al. (1984) showed that Salmonella can survive at higher levels when stored at a_w 0.43 and 0.53 compared to 0.75. We observed similar pattern of Salmonella population in both feed types. At a_w 0.43 and 0.55, Salmonella survived at higher levels in both conventional and organic feed as compared to a_w = 0.75. However, drastic decline in Salmonella populations were observed at 38°C x 0.75 at both inoculum level.

For higher inoculum level, at conditions of 11°C and 0.43, mean Salmonella populations in conventional and organic feed declined to ~10^6 and ~10^5 CFU/g of feed, respectively, (Fig. 2-1) after 80 days of storage. At conditions of 25°C x 0.43 and 38°C x 0.43, mean Salmonella populations declined to ~10^5 CFU/g in conventional feed (Fig. 2-3) and ~10^2 CFU/g in organic feed (Fig. 2-5). Similar decline in Salmonella populations were observed in conventional and organic feed, when stored at conditions of 11°C x 0.55, 25°C x 0.55, and 38°C x 0.55. For example, at conditions of 11°C x 0.55, mean Salmonella populations in conventional feed declined to ~10^6 as compared to ~10^5 CFU/g (Fig. 2-7) in organic feed. When feed was stored at conditions of 25°C x 0.55 and 38°C x 0.55, mean Salmonella populations in both conventional and organic feed declined to ~10^5 and ~10^2 CFU/g (Fig. 2-9 and 2-11) of feed, respectively. Interestingly, at conditions of 11°C x 0.75, mean Salmonella populations in both the feed types declined to ~10^6 CFU/g (Fig. 2-13) of feed after 80 days of incubation. However, at conditions of 25°C x 0.75, mean Salmonella populations in conventional feed declined to ~10^5 as compared to ~10^4 CFU/g (Fig. 2-15) of feed in organic feed over entire storage period. Mean Salmonella
populations in both the feed types declined \( \sim 10^2 \) CFU/g (Fig. 2-17) of feed, when stored at conditions of \( 38^\circ \text{C} \times 0.75 \).

For lower inoculum level, mean *Salmonella* populations in both conventional and organic feed were \( \sim 10^4 \) CFU/g of feed (Fig. 2-2) after 80 days of storage at conditions \( 11^\circ \text{C} \times 0.43 \). However, at conditions of \( 25^\circ \text{C} \times 0.43 \), mean *Salmonella* populations in conventional feed were \( \sim 10^3 \) CFU/g (Fig. 2-4) whereas in organic feed it declined to \( \sim 10^2 \) CFU/g. However, at conditions of \( 38^\circ \text{C} \times 0.43 \), mean *Salmonella* populations declined below \( 10^2 \) CFU/g (Fig. 2-6) in both the feed types. At conditions of \( 11^\circ \text{C} \times 0.55 \), mean *Salmonella* populations did not decline below \( 10^4 \) CFU/g (Fig. 2-8) of feed in conventional and organic feed. Similar phenomenon was observed, when feed was stored at \( 25^\circ \text{C} \times 0.55 \) where population did not decline below \( 10^3 \) CFU/g (Fig. 2-10) of feed in both the feed types. However, bacterial populations declined below \( 10^2 \) CFU/g (Fig. 2-12) in both conventional and organic feed, when stored at conditions of \( 38^\circ \text{C} \times 0.55 \). At storage conditions of \( 11^\circ \text{C} \times 0.75 \), mean bacterial populations in both the feed types were \( \sim 10^4 \) CFU/g (Fig. 2-14) of feed. In contrast, at conditions of \( 25^\circ \text{C} \times 0.75 \), mean *Salmonella* populations in conventional feed declined to \( \sim 10^3 \) CFU/g (Fig. 2-16) whereas bacterial population in organic feed declined below \( 10^2 \) CFU/g of feed. Interestingly, at conditions of \( 38^\circ \text{C} \times 0.75 \), mean *Salmonella* populations declined below \( 10^2 \) CFU/g (Fig. 2-18) for both conventional and organic feed.
REFERENCES


Table 2-1. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (high inoculum level) at different temperature and a$_w$ combinations

<table>
<thead>
<tr>
<th>Temp x a$_w$</th>
<th>Feed type</th>
<th><em>P</em>- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional</td>
<td>Organic</td>
</tr>
<tr>
<td>11 x 0.43</td>
<td>6.17</td>
<td>5.93</td>
</tr>
<tr>
<td>25 x 0.43</td>
<td>5.29</td>
<td>5.05</td>
</tr>
<tr>
<td>38 x 0.43</td>
<td>2.69</td>
<td>2.52</td>
</tr>
<tr>
<td>11 x 0.55</td>
<td>6.21</td>
<td>5.93</td>
</tr>
<tr>
<td>25 x 0.55</td>
<td>5.55</td>
<td>5.09</td>
</tr>
<tr>
<td>38 x 0.55</td>
<td>2.86</td>
<td>2.44</td>
</tr>
<tr>
<td>11 x 0.75</td>
<td>6.31</td>
<td>6.16</td>
</tr>
<tr>
<td>25 x 0.75</td>
<td>5.67</td>
<td>4.79</td>
</tr>
<tr>
<td>38 x 0.75</td>
<td>2.44</td>
<td>2.22</td>
</tr>
</tbody>
</table>

$^a$ Mean *Salmonella* populations (Log$_{10}$CFU/g) of three replicates per temperature and a$_w$ combination for two replicated experiments.

$^b$ *P*-value were considered significant at <0.05.
Table 2-2. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (low inoculum level) at different temperature and $a_w$ combinations$^a$.

<table>
<thead>
<tr>
<th>Temp x $a_w$</th>
<th>Feed type</th>
<th>P-value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional</td>
<td>Organic</td>
</tr>
<tr>
<td>11 x 0.43</td>
<td>4.29</td>
<td>4.12</td>
</tr>
<tr>
<td>25 x 0.43</td>
<td>3.26</td>
<td>2.64</td>
</tr>
<tr>
<td>38 x 0.43</td>
<td>1.10</td>
<td>0.80</td>
</tr>
<tr>
<td>11 x 0.55</td>
<td>4.58</td>
<td>4.24</td>
</tr>
<tr>
<td>25 x 0.55</td>
<td>3.89</td>
<td>3.15</td>
</tr>
<tr>
<td>38 x 0.55</td>
<td>1.39</td>
<td>1.06</td>
</tr>
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<td>11 x 0.75</td>
<td>4.43</td>
<td>4.08</td>
</tr>
<tr>
<td>25 x 0.75</td>
<td>2.96</td>
<td>1.86</td>
</tr>
<tr>
<td>38 x 0.75</td>
<td>0.93</td>
<td>0.81</td>
</tr>
</tbody>
</table>

$^a$ Mean *Salmonella* populations (Log$_{10}$CFU/g) of three replicates per temperature and $a_w$ combination for two replicated experiments.

$^b$ P-value were considered significant at <0.05.
Table 2-3. Composition of ingredients in organic broiler-grower feed

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein (min)</td>
<td>15.00</td>
</tr>
<tr>
<td>Lysine (min)</td>
<td>0.70</td>
</tr>
<tr>
<td>Methionine (min)</td>
<td>0.30</td>
</tr>
<tr>
<td>Crude Fat (min)</td>
<td>5.00</td>
</tr>
<tr>
<td>Crude Fiber (max)</td>
<td>5.00</td>
</tr>
<tr>
<td>Calcium (Ca) (min)</td>
<td>0.60</td>
</tr>
<tr>
<td>Calcium (Ca) (max)</td>
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</tr>
<tr>
<td>Phosphorus (P) (min)</td>
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</tr>
<tr>
<td>Salt (NaCl) (min)</td>
<td>0.30</td>
</tr>
<tr>
<td>Salt (NaCl) (max)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*Percentage of ingredients in 100 g of feed.

**INGREDIENTS:**
### Table 2-4. Composition of ingredients in conventional broiler-grower feed

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein (min)</td>
<td>15.00</td>
</tr>
<tr>
<td>Lysine (min)</td>
<td>0.65</td>
</tr>
<tr>
<td>Methionine (min)</td>
<td>0.29</td>
</tr>
<tr>
<td>Crude Fat (min)</td>
<td>2.70</td>
</tr>
<tr>
<td>Crude Fiber (max)</td>
<td>5.00</td>
</tr>
<tr>
<td>Calcium (Ca) (min)</td>
<td>0.60</td>
</tr>
<tr>
<td>Calcium (Ca) (max)</td>
<td>1.10</td>
</tr>
<tr>
<td>Phosphorus (P) (min)</td>
<td>0.60</td>
</tr>
<tr>
<td>Salt (NaCl) (min)</td>
<td>0.20</td>
</tr>
<tr>
<td>Salt (NaCl) (max)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*Percentage of ingredients in 100 g of feed*

Ingredients: Grain Products, Processed Grain By-Products, Plant Protein Products, Animal Protein Products, Calcium carbonate, Molasses Products, Salt, Methionine Supplement, Choline Chloride, Vitamin E Supplement, L-Lysine, Menadione Dimethylpyrimidinol Bisulfite, Riboflavin Supplement, Vitamin A Supplement, Vitamin B-12 Supplement, Pyridoxine Hydrochloride, Niacin Supplement, Folic Acid, Calcium Pantothenate, Biotin, Vitamin D3 Supplement, Manganese Oxide, Zinc Oxide, Copper Sulfate, and Calcium Iodate.
Fig. 2-1. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 11°C and $a_w = 0.43$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-2. Mean *Salmonella* populations (Log$_{10}$ CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 11°C and $a_w = 0.43$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-3. Mean *Salmonella* populations (Log\(_{10}\) CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 25\(\,^{\circ}\)C and \(a_w\) = 0.43). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-4. Mean *Salmonella* populations (Log$_{10}$ CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 25°C and $a_w = 0.43$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-5. Mean *Salmonella* populations (Log$_{10}$ CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 38°C and $a_w = 0.43$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-6. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 38°C and $a_w = 0.43$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-7. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 11°C and $a_w = 0.55$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-8. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 11°C and $a_w = 0.55$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-9. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 25°C and $a_w = 0.55$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-10. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 25°C and $a_w = 0.55$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-11. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 38°C and $a_w = 0.55$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-12. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 38°C and $a_w = 0.55$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-13. Mean *Salmonella* populations (Log_{10} CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 11°C and a_w = 0.75). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-14. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 11°C and $a_w = 0.75$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-15. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 25°C and $a_w = 0.75$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-16. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 25°C and $a_w = 0.75$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-17. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (high inoculum level) over storage period of 80 days at conditions (temperature = 38°C and $a_w = 0.75$). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
Fig. 2-18. Mean *Salmonella* populations (Log$_{10}$CFU/g) in conventional and organic poultry feed (low inoculum level) over storage period of 80 days at conditions (temperature = 38°C and $a_w$ = 0.75). Each data point represents mean of three replicates for two experiments (6 observations total) per treatment.
CHAPTER 3
CONCLUSION

The purpose of this study was to determine the survivability of Salmonella in conventional versus organic poultry feed at different temperature and \( a_w \) combinations. The result of this study showed that mean Salmonella populations in organic feed were significantly lower than conventional feed for the majority of temperature-by-\( a_w \) combinations at both inoculum levels over a period of 80 days. Our observation revealed, when high inoculated feed was stored at conditions of 11\(^\circ\)C under the three different \( a_w \), statistically significant differences (P<0.05) were observed. In contrast, no significant differences were observed at storage temperature 38\(^\circ\)C. Likewise, when feed was stored at conditions of 25\(^\circ\)C x 0.55 and 25\(^\circ\)C x 0.75 Salmonella populations were statistically significantly different, except for 25\(^\circ\)C x 0.43. In spite of statistically significant differences between the two feed types at 11\(^\circ\)C x 0.75 and 25\(^\circ\)C x 0.55, however; the difference in the mean was too small (<1.0 log) to be considered biologically meaningful. For the low inoculum, significant differences (P<0.05) between the two feed types were observed at all storage temperature and \( a_w \) combinations. The mean Salmonella populations in organic feed were lower than conventional feed for all temperature-by-\( a_w \) combinations. Although, statistically significant differences were observed between the conventional and organic feed types in the mean Salmonella populations for the following temperature-by-\( a_w \) combinations: 11\(^\circ\)C x 0.43, 11\(^\circ\)C x 0.55, 11\(^\circ\)C x 0.75, 25\(^\circ\)C x 0.55, 38\(^\circ\)C x 0.55, and 38\(^\circ\)C x 0.75; however, the difference in the mean was too small (<1.0 log) to be considered biologically meaningful. Based on these findings, there were no biologically significant reductions in Salmonella populations observed at both inoculum levels in organic broiler feed compared to
conventional feed, however; significant reductions were observed in proportion of feed samples positive for *Salmonella* in organic feed as compared to conventional feed at both inocula.