Survival of *Salmonella enterica* in poultry feed is strain dependent

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ABSTRACT Feed components have low water activity, making bacterial survival difficult. The mechanisms of *Salmonella* survival in feed and subsequent colonization of poultry are unknown. The purpose of this research was to compare the ability of *Salmonella* serovars and strains to survive in broiler feed and to evaluate molecular mechanisms associated with survival and colonization by measuring the expression of genes associated with colonization (*hilA*, *invA*) and survival via fatty acid synthesis (*cfa*, *fabA*, *fabB*, *fabD*). Feed was inoculated with 1 of 15 strains of *Salmonella enterica* consisting of 11 serovars (Typhimurium, Enteriditis, Kentucky, Seftenburg, Heidelberg, Mbandanka, Newport, Bairely, Javiana, Montevideo, and Infantis). To inoculate feed, cultures were suspended in PBS and survival was evaluated by plating samples onto XLT4 agar plates at specific time points (0 h, 4 h, 8 h, 24 h, 4 d, and 7 d). To evaluate gene expression, RNA was extracted from the samples at the specific time points (0, 4, 8, and 24 h) and gene expression measured with real-time PCR. The largest reduction in *Salmonella* occurred at the first and third sampling time points (4 h and 4 d) with the average reductions being 1.9 and 1.6 log cfu per g, respectively. For the remaining time points (8 h, 24 h, and 7 d), the average reduction was less than 1 log cfu per g (0.6, 0.4, and 0.6, respectively). Most strains upregulated *cfa* (cyclopropane fatty acid synthesis) within 8 h, which would modify the fluidity of the cell wall to aid in survival. There was a weak negative correlation between survival and virulence gene expression indicating downregulation to focus energy on other gene expression efforts such as survival-related genes. These data indicate the ability of strains to survive over time in poultry feed was strain dependent and that upregulation of cyclopropane fatty acid synthesis and downregulation of virulence genes were associated with a response to desiccation stress.

Key words: *Salmonella*, poultry feed, virulence, survival, gene regulation

2014 Poultry Science 93:441–447
http://dx.doi.org/10.3382/ps.2013-03401

INTRODUCTION

Each year, 31 identified pathogens cause an estimated 9.4 million episodes of foodborne illness in the United States (Scallan et al., 2011). Among these foodborne pathogens, nontyphoidal *Salmonella enterica* is the leading cause of death and hospitalizations (Scallan et al., 2011). Foodborne pathogens can be acquired by food-producing animals, which may transmit zoonotic pathogens through the food chain and subsequently cause human foodborne illness (Crump et al., 2002). Poultry and poultry products are the leading source of non-Typhi serotypes of *S. enterica* in the United States (Braden, 2006). Poultry may be colonized with *S. enterica* but not cause any signs or symptoms of disease in the birds. Thus, if intestinal contents are released during processing, contamination of the carcasses may occur (Rigby et al., 1980).

The initial source of *S. enterica* to the birds can be transmitted from several vectors (Jarquin et al., 2009). Protein and by-product ingredients originating from animals that are used in feed have been suggested as a source of *S. enterica* (Williams, 1981; Davies et al., 2004). Given the conditions of the source of the main ingredients, and processing, transportation, and storage, poultry feed has a higher potential than other sources to become contaminated with *S. enterica* (Jones, 2011).

Currently, *S. enterica* serovar Kentucky is the dominant serovar isolated from poultry and poultry products in the United States (Foley et al., 2008), but this serovar rarely causes foodborne illness. Conversely, even though isolation of serovar Enteritidis from poultry products has declined, infections with this serovar have increased (CDC, 2010). Thus, it appears that survival on the farm and in other poultry-related environments including feed may not be related to the ability of *S. enterica* to cause disease (Foley et al., 2008). Therefore, the main objective of this study was to compare the

©2014 Poultry Science Association Inc.
Received June 10, 2013.
Accepted October 15, 2013.
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Table 1. A table of the *Salmonella enterica* serovars, the source of the strains, and references describing characteristics of the strains used in this work

Salmonella enterica serovar	Source	Reference
Typhimurium DT104	Human infection	Threlfall, 2000
Typhimurium ATCC 23595 (LT2)	Laboratory strain	Swords et al., 1997
Typhimurium ATCC 14028	Laboratory strain	None
Enteritidis (WT)	Human infection	None
Enteritidis ATCC 13076	Human infection	None
Kentucky	Poultry carcass	Clement et al., 2010
Kentucky	Poultry carcass	Clement et al., 2010
Seftenburg	Poultry farm	Rodriguez et al., 2006
Heidelberg	Poultry farm	Rodriguez et al., 2006
Mbandanka	Poultry carcass	Melendez et al., 2010
Newport	Poultry carcass	Melendez et al., 2010
Bairely	Poultry carcass	Melendez et al., 2010
Javana	Poultry farm	Rodriguez et al., 2006
Montevideo	Swine farm	Rodriguez et al., 2006
Infantis	Poultry farm	Rodriguez et al., 2006

survival capabilities of *S. enterica* serovars and strains in broiler feed over time in storage. A second objective was to investigate molecular mechanisms associated with survival and virulence by evaluating expression of specific genes associated with these characteristics.

MATERIALS AND METHODS

Bacteria and Culturing Conditions

In these studies a total of 11 serovars consisting of 15 strains of *S. enterica* were used (Table 1). All *S. enterica* strains were initially cultured on tryptic soy agar (Becton, Dickinson and Company, Sparks, MD) and incubated at 37°C for 24 h. After incubation, a 10-μL loop of culture was inoculated into 30 mL of tryptic soy broth (Becton, Dickinson and Company; pH 7.2) and incubated in a shaking water bath at 37°C for 15 h. From this culture, 1 mL was inoculated into tryptic soy broth and incubated in a shaking water bath at 37°C for 3 h. The culture then was centrifuged at 8,000 × *g* for 5 min and the supernatant discarded. The culture was washed 3 times by resuspending the pellet in PBS (Becton, Dickinson and Company), centrifuging (8,000 × *g* for 5 min at 25°C), and finally resuspending in PBS. *Salmonella* suspensions were standardized to 0.15 at 630 nm by spectrophotometry so that all serovars were used at approximately the same concentrations (7 log cfu·mL^{-1}). A dilution series was also conducted on the suspension to precisely determine the initial *S. enterica* concentration.

Spiking and Analysis of Feed Sample

A Chick Starter/Grower-AMP BMD feed was purchased from a local co-op (Knoxville, TN) and sieved through a screen (no. 8; 2.38-mm openings) to remove dust and small particles. The composition of the formulated starter feed is presented in Table 2. Water activity of the feed was measured using a water activity meter (Aqua Lab, Decagon Services Inc., Pullman, WA). For the survival studies, 10-μL aliquots of the *S. enterica* suspension prepared as described in the previous section were placed into 2 g of the feed in 5-mL tubes and mixed by agitation. The inoculated feed was stored at 25°C. At specific time points (0, 4, 8, and 24 h, and 4 and 7 d), *S. enterica* survival was evaluated using standard microbiological methods and a standard dilution series. We chose to use 7 d because this is the average time of storage of poultry feed on poultry farms. Briefly, the sample was suspended in 2 mL of PBS, vortexed, and a 100-μL portion of the solution was used in a dilution series that was inoculated on XLT4 (xylose lysine tergitol-4, Becton, Dickinson and Company) agar, which was incubated at 37°C for 24 h. An uninoculated sample of the poultry feed acted as the negative control. Triplicate samples were evaluated with 2 repetitions performed for each serovar.

Table 2. Formulation and ingredient list[1] of the starter/grower feed (co-op chick) used in this study

Component	Guaranteed analysis, %
CP	19
Lysine	0.82
Methionine	0.27
Crude fat	3.5
Crude fiber	4.5
Calcium	0.80–1.30
Phosphorus	0.7
Salt	0.25–0.75
Active drug ingredient (g/t)	
Amprolium	125.11
Bactracin methylene disalicylate	220.46

[1]Ingredients: grain products, plant protein products, processed grain by-products, molasses products, propionic acid, calcium carbonate, calcium phosphate, salt, choline chloride, yucca schidegera extract, *Bacillus subtilis*, niacin supplement, vitamin E supplement, calcium pantothenate, riboflavin supplement, vitamin A acetate, menadione dimethylpyrimidinol bisulfite, vitamin D_3 supplement, biotin, vitamin B_{12} supplement, pyridoxine hydrochloride, folic acid, thiamine, ferrous sulfate, manganous oxide, zinc oxide, copper oxide, calcium iodate, sodium selenite, cobalt carbonate.

Table 3. A list of the genes, primer sequences, and references for the primers that were used to evaluate gene expression changes of *Salmonella enterica* strains used in this study

Target gene[1]	Sequence (5′ to 3′)	Reference
16S	Forward: GCGGCCCCCTGGACAAAGAC	Gonzalez-Gil et al., 2012
	Reverse: TAGCTCCGGAAGCCACGCCT	
hilA	Forward: ATGCCATAGCATTTTTATCC	Park et al., 2011
	Reverse: GATTTAATCTGTATCAGG	
invA	Forward: CTGTCTGGCGGTGACGCTGG	Own design. NCBI[2] reference
	Reverse: ACGCGCCATTGCTCCACGAA	Sequence: NC_003198.1
cfa	Forward: GCTGGTGGGAATGCGAGCGT	Own design. NCBI reference
	Reverse: CAGCACACGCATCCCCGGTT	Sequence: NC_011294.1
fabA	Forward: ACTCCCTGCGCCGAACATGC	Own design. NCBI reference
	Reverse: CACTTCGCCCACGCCCAGAG	Sequence: NC_011294.1
fabB	Forward: CCGCGTGGTCTGAAAGCCGT	Own design. NCBI reference
	Reverse: GGACAGTGCGCCCATCGCAT	Sequence: NC_011294.1
fabD	Forward: ACCCAGCAAGGTCCAGCGG	Own design. NCBI reference
	Reverse: TTCGCGCCAGCGGCTTTACA	Sequence: NC_011294.1

[1]*16S* = housekeeping gene; *hilA* and *invA* = genes involved in virulence and colonization; *cfa* = cyclopropane fatty acid gene; *fabA*, *fabB*, and *fabD* = fatty acid biosynthesis genes.

[2]NCBI = National Center for Biotechnology Information.

RNA Preparation

Total RNA was isolated from the samples as described by Gonzalez-Gil et al. (2012) with some modification. At specific time points (0, 4, 8, and 24 h) and equal volume of RNA protect bacterial reagent (Qiagen, Valencia, CA) was added to a 2-mL microfuge tube containing the *Salmonella* feed suspensions and allowed to stand at room temperature for 5 min. Subsequently, RNA was extracted from the samples using the RNeasy mini kit (Qiagen) as directed by the manufacturer. After extraction, the RNA samples were subjected to a DNase treatment utilizing the Qiagen DNase kit (Qiagen) as directed by the manufacturer. All samples then were quantified using spectrophotometry (Nanodrop ND-1000, ThermoScientific, Pittsburgh, PA).

Quantitative Reverse-Transcription Real-Time PCR

After purification, cDNA was synthesized from the RNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). All quantitative reverse-transcription real-time PCR (**qRT-PCR**) reactions were performed as described by Gonzalez-Gil et al. (2012) using the ABI 7100 RT-PCR system (Applied Biosystems, Carlsbad, CA). Briefly, a 20-μL total volume consisted of 10 μL of Power SYBR Green PCR Master Mix (Life Technologies Corporation, Carlsbad, CA), 300 n*M* of each primer, 100 ng of cDNA template, and water to volume. With the exception of *hilA* and 16S rRNA, primers were designed using the NCBI Primer-BLAST tool and evaluated for specificity (Table 3). All primers were synthesized by Integrated DNA Technologies (Coralville, IA). The qRT-PCR reactions were optimized to the conditions of 95°C for 15 min for the initial activation of Taq polymerase. This was followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and amplification at 60°C for 30 s with fluorescence being measured during the extension phase. Melting curves were conducted subsequently and consisted of 95°C for 15 s, 60°C for 5 min to a final temperature of 95°C for 15 s. All reactions were performed independently and in triplicate.

Analysis of Gene Expression

Samples were normalized using the 16S rRNA gene as an internal standard (Table 3). The relative changes (n-fold) in gene expression between samples were calculated using the $2^{-\Delta\Delta C(T)}$ method as described by Livak and Schmittgen (2001). Fold change in expression for specific target gene was determined and these data were used to generate heat maps within a Microsoft Excel 14.3.5 (Microsoft Corporation, Redmond, WA) spreadsheet using the conditional formatting and color scale functions.

Statistical Analysis

For survival and water activity experiments, each strain was sampled in duplicate with triplicate repetitions, and culturable cfu counts were analyzed via mixed ANOVA analysis ($P < 0.05$) to determine statistical differences between strains. Results are expressed as least squares means with SEM. For water activity measurements, each strain was sampled in triplicate for each time point and analyzed as above for the survival experiments. The software used was SAS 9.3 (SAS Institute Inc., Cary, NC).

RESULTS

The water activity of the sample of spiked feed was measured at specific times of 0, 4, 8, 24 h, and 4 and 7 d (Table 4). This was done to correlate water activity in the feed with any impact on the survival of *S. enterica*. Not surprisingly, there was some correlation

Table 4. Measurement of water activity (a_w) in the poultry feed, before being spiked with *Salmonella enterica* cultures, and after spiking at specific time points[1]

Sample	a_w
Unspiked	0.35 ± 0.001^{a}
0 h	0.74 ± 0.001^{b}
4 h	0.70 ± 0.003^{c}
8 h	0.69 ± 0.003^{d}
24 h	0.67 ± 0.001^{e}
4 d	0.65 ± 0.002^{f}
7 d	0.61 ± 0.001^{g}

[a–g]Mean values within a column that do not have the same superscript letter are significantly different ($P < 0.05$).

[1]Values of SEM ± from triplicates from each *S. enterica* strain.

between the water activity in the spiked feed and the survival rates of the bacteria. Water activity consistently decreased over the course of the experiments as did the counts of culturable *S. enterica*. However, the correlation coefficients indicated that there was no significant correlation between water activity and reduction in culturable *Salmonella*. This is most likely due to the large variation in reduction of *Salmonella* counts between each time point.

The culturable *S. enterica* populations (log $cfu \cdot g^{-1}$) were determined at 0, 4, 8, and 24 h, and 4 and 7 d, and differences in the survival of the bacteria were found to be dependent on serovar and strain (Table 5). After 7 d, almost 3 logs (cfu per g of feed) of *Salmonella* Enteriditis (WT) and *Salmonella* Typhimurium ATCC 23595 (LT2) were recovered from the feed samples. After 4 d of incubation at room temperature, *Salmonella* Typhimurium 14028 and *Salmonella* Montevideo could not be recovered. Both strains of *Salmonella* Kentucky and *Salmonella* Typhimurium 14028 had the most rapid decrease after 4 h with approximately 3 logs (cfu per g of feed) less than the initial inoculum recovered from the feed. Both strains of *Salmonella* Enteritidis, *Salmonella* Seftenburg, *Salmonella* Mbandanka, and *Salmonella* Infantis had the lowest decrease (approximately 1 log $cfu \cdot g^{-1}$) in recoverable bacteria after 4 h. The remaining strains decreased by approximately 2 log $cfu \cdot g^{-1}$ from the initial inoculum levels after 4 h of incubation at room temperature. Interestingly, data regarding strains of the same serovar were quite variable. The 3 Typhimurium strains had different patterns in reduction of *Salmonella*, whereas the Kentucky and Enteritidis strains had similar patterns when comparing data of the same serovar.

Relative fold change in gene expression for each gene was calculated and heat maps generated for the 3 time points sampled over the course of the experiment (Figure 1). These maps then were sorted from ascending to descending for each gene. In this way, it was visually apparent that the *cfa* gene was upregulated in most serovars after 4 h. Furthermore, it appeared that there was a correlation between regulation of the *cfa* gene and the *fabB* gene at the 8 and 24 h time points (0.93 and 0.90, respectively). There were no other apparent

Table 5. Changes in the counts of culturable *Salmonella enterica* serovars (cfu/g of feed) expressed in log recovered from artificially inoculated feed at specific time points

	Changes between time points[1]				
Strain	0 h to 4 h	4 h to 8 h	8 h to 24 h	24 h to 4 d	4 d to 7 d
Salmonella Typhimurium DT104	2.17 ± 0.10^{a}	0.38 ± 0.10^{bc}	0.51 ± 0.12^{b}	2.71 ± 0.49^{a}	-0.58 ± 0.78^{abcd}
Salmonella Typhimurium ATCC 23595 (LT2)	1.79 ± 0.11^{ab}	0.03 ± 0.14^{bc}	0.83 ± 0.16^{bc}	0.73 ± 0.27^{ab}	-0.22 ± 0.28^{d}
Salmonella Typhimurium ATCC 14028	3.47 ± 0.80^{abc}	-0.15 ± 0.80^{abc}	1.59 ± 0.29^{a}	1.42 ± 0.45^{b}	NC $\pm 0.00^{d}$
Salmonella Enteritidis (WT)	1.40 ± 0.10^{bc}	0.13 ± 0.05^{c}	0.55 ± 0.09^{b}	1.19 ± 0.14^{a}	0.42 ± 0.10^{cd}
Salmonella Enteritidis ATCC 13076	1.03 ± 0.05^{c}	0.74 ± 0.28^{abc}	0.29 ± 0.17^{bcd}	1.50 ± 0.06^{a}	2.10 ± 0.00^{a}
Salmonella Kentucky A	3.01 ± 0.81^{abc}	0.36 ± 1.07^{abc}	0.69 ± 0.74^{abc}	0.75 ± 0.44^{ab}	0.7 ± 0.44^{bcd}
Salmonella Kentucky F	2.95 ± 0.47^{ab}	0.20 ± 0.64^{bc}	0.92 ± 0.75^{abc}	0.00 ± 0.94^{ab}	0.35 ± 0.65^{abcd}
Salmonella Seftenburg	0.97 ± 0.21^{abc}	-0.22 ± 0.27^{abc}	0.38 ± 0.24^{bcd}	3.09 ± 0.47^{a}	1.05 ± 0.47^{abcd}
Salmonella Heidelburg	1.57 ± 0.35^{abc}	1.28 ± 0.11^{a}	-0.38 ± 0.09^{d}	1.75 ± 0.54^{ab}	1.42 ± 0.64^{b}
Salmonella Mbandanka	1.35 ± 0.14^{bc}	0.59 ± 0.11^{b}	-0.02 ± 0.08^{cd}	2.21 ± 0.62^{ab}	0.33 ± 0.72^{abcd}
Salmonella Newport	2.30 ± 0.27^{abc}	0.85 ± 0.24^{abc}	0.87 ± 0.11^{b}	1.15 ± 0.43^{ab}	1.41 ± 0.64^{abcd}
Salmonella Bairely	1.97 ± 0.20^{abc}	0.43 ± 0.20^{abc}	0.18 ± 0.21^{bcd}	0.94 ± 0.22^{ab}	2.02 ± 0.29^{ab}
Salmonella Javiana	2.09 ± 0.32^{abc}	0.77 ± 0.35^{abc}	0.44 ± 0.05^{b}	1.42 ± 0.60^{ab}	1.94 ± 0.63^{abcd}
Salmonella Montevideo	1.94 ± 0.27^{abc}	0.93 ± 0.49^{abc}	1.21 ± 0.71^{abcd}	2.16 ± 0.68^{ab}	NC $\pm 0.00^{d}$
Salmonella Infantis	0.82 ± 0.16^{c}	0.71 ± 0.20^{abc}	0.40 ± 0.11^{bc}	2.14 ± 0.43^{a}	1.75 ± 0.5^{abcd}

[a–d]Mean values within a column that do not have the same superscript letter are significantly different ($P < 0.05$).

[1]Values ± SEM from triplicates with duplicate repetition samples. NC: no change between time points.

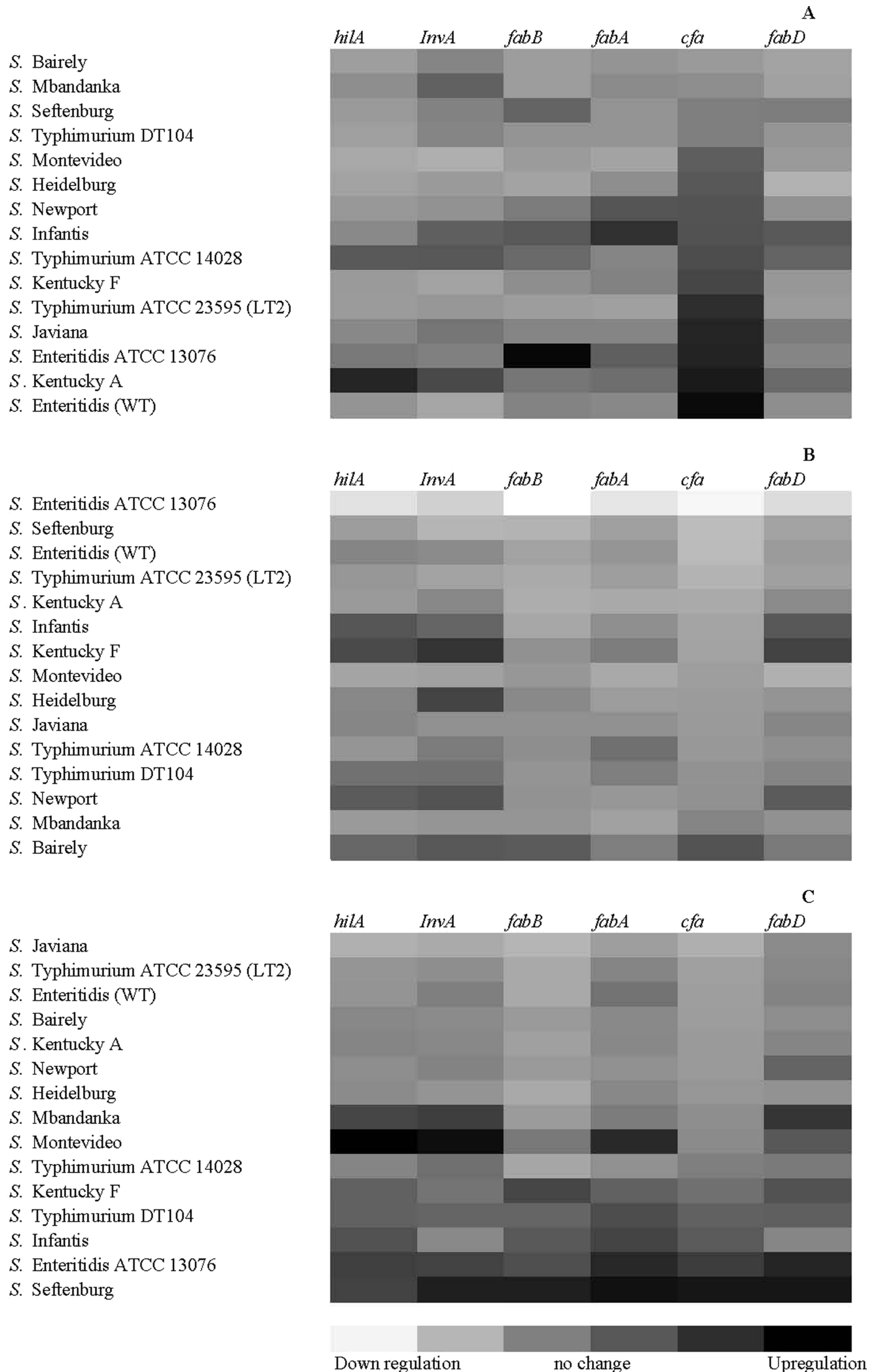

Figure 1. A heat map of relative fold change in gene expression of genes involved in virulence and colonization (*hilA*, *InvA*) and fatty acid synthesis (*cfa*, *fabB*, *fadD*, *fabA*) in 15 *Salmonella enterica* serovars artificially inoculated into poultry feed and sampled after incubation at room temperature at 4 h (panel A), 8 h (panel B), and 24 h (panel C). Maps are sorted based on the *cfa* gene in ascending order of regulation for each time point. *cfa* = cyclopropane fatty acid gene; *fabA*, *fabB*, and *fabD* = fatty acid biosynthesis genes.

gene regulation and gene correlations consistent among all strains.

Correlation analysis was performed to determine if survival of the *S. enterica* serovars was correlated to expression of specific genes. A low positive coefficient of correlation was obtained between bacterial survival and the genes *cfa*, *fabA*, and *fabB* (0.23, 0.04, and 0.13, respectively). For the genes *invA*, *fabD*, and *hilA*, a low negative correlation (−0.24, −0.04, and −0.28) was correlated with the survival capability of the *S. enterica* strains tested. Although the values of correlation were numerically different, they were not statistically significant ($P > 0.05$).

DISCUSSION

According to Ha et al. (1998), *S. enterica* survival in feed can vary and is dependent on formulation. In their study, Ha et al. (1998) also found that aerobic bacterial counts recovered from feeds containing meat and bone meal were greater than those containing soybean meals. However, Pektar et al. (2011) reported that there were no differences in the abilities of *S. enterica* to survive in conventional versus organic feed where the conventional feed contained bone and poultry meal, which was replaced in the organic feed with alfalfa meal. *Salmonella enterica* contamination on individual ingredients of the feed is also an important fact to consider, because *S. enterica* has been isolated from feed ingredients including, grains, oilseed meal, feather and fish meal, and meat by-products (Maciorowski et al., 2004).

Survival of *S. enterica* in low water activity foods is well documented (Tamminga et al., 1976; Juven et al., 1984; Rowe et al., 1987; Lehmacher et al., 1995; Beuchat, 2009). Interestingly, previous studies suggest that *S. enterica* survival is higher in foods with water activity (**a_w**) between 0.43 and 0.55 than foods at an a_w of 0.75 (Juven et al., 1984; Pektar et al., 2011). Because water activity did not drop below 0.61 in this study, water activity may have been suboptimal for the *S. enterica* strains we evaluated for survival in feed.

The *invA* gene allows *Salmonella* to enter epithelial cells, playing an important role in the invasion and disease process (Galán et al., 1992). The second virulence gene evaluated in this study, *hilA*, regulates the expression of invasion genes in response to environmental stimuli including osmolarity, oxygen levels, and pH (Durant et al., 2000; Fluit, 2005; Chuanchuen et al., 2010; Park et al., 2011; Gonzalez-Gil et al., 2012). In the present study, there was an overall negative correlation between survival and upregulation of these 2 genes indicating that perhaps efforts for virulence were shifted away from these genes and instead focused on upregulation of stress responses (Gonzalez-Gil et al., 2012).

To survive the stress of desiccation, some bacteria increase membrane fluidity (Baysse and O'Gara, 2007). For *S. enterica*, membrane fluidity can be modified with an increase in de novo synthesis of unsaturated fatty acids, which occurs via the *fabA*-*fabB* pathway. Likewise, the *cfa* gene encodes cyclopropane fatty acid (**CFA**) synthase, an enzyme that cyclizes UFA to improve membrane fluidity (Kim et al., 2005). Conversely, *fabD* is activated to produce saturated fatty acids, which decrease membrane fluidity. Thus, the upregulation of *cfa* in this study at the 4-h time point was not surprising as an increase in CFA is considered to be an indicator of starvation or desiccation stress (Kieft et al., 1994).

Low water activity food products can become cross contaminated after processing by factors including poor sanitization practices, poor equipment design, and poor ingredient control, which presents a significant food safety risk (Podolak et al., 2010). Some research indicates the infectious dose of *S. enterica* is lower when infection occurs via a contaminated low a_w food (Rowe et al., 1987; Greenwood and Hooper, 1983). The reason for this is not exactly known. However, data from this study indicate that this may not be due to upregulation of virulence-associated genes *hilA* and *invA* because our data showed a tendency for these genes to be downregulated in lower water activity. Instead, the lower infectious dose may be an adaptive tolerance response where cells that survived the low water activity are more stress resistant, making it easier for these cells to survive the subsequent stress of passage through the acidic gastrointestinal environment (Ma et al., 2009). It has also been suggested that pathogens in low water activity foods are typically metabolically inactive, and this metabolic state makes the cells less susceptible to stresses such as those encountered in the gastrointestinal environment (Barat et al., 2012).

The data indicate that differences in survival and gene expression vary by serovars of *S. enterica*, caution should be taken if applying the results of this study to other serovars of *S. enterica* that have not been evaluated. In addition, because only one type of feed and incubation temperature were used, additional experiments are necessary to understand how these variables may affect the results. In conclusion, this study demonstrated that the ability of *S. enterica* to survive over storage time in poultry feed was serovar and strain dependent. Furthermore, the data indicate that the upregulation of short chain fatty acid synthesis and downregulation of virulence genes may be associated with survival in the poultry feed component.

ACKNOWLEDGMENTS

This study was funded by a HATCH grant awarded to I. Hanning by the University of Tennessee Experiment Station.

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