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Research Note

Evaluating Chemical Mitigation of *Salmonella* Typhimurium ATCC 14028 in Animal Feed Ingredients

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ABSTRACT

Salmonella Typhimurium is a potential feed safety hazard in animal feed ingredients. Thermal mitigation of *Salmonella* spp. during rendering is effective but does not eliminate the potential for cross-contamination. Therefore, the objective of this experiment was to evaluate the effectiveness of chemicals to mitigate postrendering *Salmonella* Typhimurium ATCC 14028 contamination in rendered proteins over time. Treatments were arranged in a 6×4 factorial with six chemical treatments and four rendered protein meals. The chemical treatments included (i) control without chemical treatment, (ii) 0.3% commercial formaldehyde product, (iii) 2% essential oil blend, (iv) 2% medium chain fatty acid blend, (v) 3% organic acid blend, and (vi) 1% sodium bisulfate. The four rendered protein meals included (i) feather meal, (ii) blood meal, (iii) meat and bone meal, and (iv) poultry by-product meal. After matrices were chemically treated, they were inoculated with *Salmonella* Typhimurium ATCC 14028, stored at room temperature, and enumerated via plate counts on days 0, 1, 3, 7, 14, 21, and 42 postinoculation. The *Salmonella* concentration in ingredients treated with medium chain fatty acid and commercial formaldehyde were similar to one another ($P = 0.23$) but were 2 log lower than the control ($P < 0.05$). Ingredients treated with organic acids and essential oils also had lower *Salmonella* concentrations than the control ($P < 0.05$). Time also played a significant role in *Salmonella* mitigation, because all days except days 14 and 21 ($P = 0.92$) differed from one another. Rendered protein matrix also affected *Salmonella* stability, because concentrations in meat and bone meal and blood meal were similar to one another ($P = 0.36$) but were greater than levels in feather meal and poultry by-product meal ($P < 0.05$). In summary, chemical treatment and time both mitigated *Salmonella* Typhimurium ATCC 14028, but their effectiveness was matrix dependent. Time and chemical treatment with medium chain fatty acids or a commercial formaldehyde product were most effective at mitigating *Salmonella* Typhimurium ATCC 14028 in rendered protein meals.

Key words: Animal feed; Chemical treatment; Feed safety; *Salmonella*

Salmonella spp. cross-contamination of ingredients is a major concern in the feed and rendering industries. In the United States alone, 11.2×10^9 lb (ca. 5.1×10^9 kg) of protein and 10.9×10^9 lb (ca. 4.9×10^9 kg) of fat are produced each year, of which 85% is used in animal feed ingredients *(18)*. The first documented case of *Salmonella* spp. contamination in animal feed was as far back as 1948 *(11)*. Due to the historical occurrence of *Salmonella* spp. in animal feed, the U.S. Food and Drug Administration (FDA) carried out surveys of pathogen contamination in animal-based rendering plants across the United States. Of the 101 animal-based protein samples collected in 1993, 56% tested positive for *Salmonella enterica (21)*. As a follow-up, finished feed samples from feed mills and on-site farms were tested in 1994, and the FDA reported that 25% of the 89 samples tested were positive for *S. enterica (21)*. Since then, other studies have shown similar results, including one in which 85% of 165 samples tested were positive for gram-negative bacteria and 10% were positive for *Salmonella* spp. *(13)*. Although *Salmonella* spp. may be perceived as a lower risk hazard in animal feed, salmonellosis of animals has been linked to human illness *(9)*. If *Salmonella* spp. contamination exists in animal feed or ingredients, it should be mitigated to minimize the risk to animal or human health.

Potential methods of bacterial contaminant mitigation can be characterized as thermal or nonthermal in nature. Whereas thermal mitigation is an attractive option because it does not require the introduction of foreign compounds, it is a point-in-time strategy that does not eliminate the chance for recontamination *(16)*. For example, Binter et al. *(3)* demonstrated that up to 86% of thermally processed samples collected from pellet coolers tested positive for *Salmonella*. Alternatively, nonthermal mitigation methods may include the use of chemicals, such as organic acids (OA), formaldehyde, medium-chain fatty acids (MCFA), essential oils (EO), and sodium bisulfate *(8, 16, 19)*. Of these, the most common feed additive is OA, particularly propionic, formic, lactic, and acetic acids. All of these OA have been shown to be effective at reducing the concentration of *Salmonella* spp. *(1, 2, 20, 22)*. Another chemical additive that is approved for the mitigation of *Salmonella* spp. in

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animal feed is 0.03% formaldehyde *(26)*. Some EO, such as oregano and rosemary oils, have also been used to mitigate *Salmonella* spp., reducing the bacterial load by 1 to 2 log CFU/g in food products *(12, 24)*. MCFA, such as caprylic and capric acid, have also been shown to be potential *Salmonella* spp. mitigants that act by damaging the cell membrane of the bacteria *(17)*. Although some data are available on the mitigation potential of particular chemicals against *Salmonella* spp. inocula, very little research has evaluated the ability of chemical treatment of various feed ingredients to prevent cross-contamination with the bacteria. Because various physical states, nutrient composition, and properties of each chemical additive and feed matrix are different, each chemical may interact differently as a mitigant. Therefore, the objective of this experiment was to evaluate the effectiveness of various chemical treatments to mitigate postprocessing *Salmonella* Typhimurium ATCC 14028 contamination in feed ingredients.

MATERIALS AND METHODS

Chemical treatment. Six chemical treatments were applied to four different feed matrices. The chemical treatments included (i) *Salmonella* Typhimurium ATCC 14028 positive with no chemical addition, (ii) 0.3% (wt/wt) commercial formaldehyde product (Termin-8, Anitox Corp, Lawrenceville, GA), (iii) 2% (wt/wt) EO blend (1:1 ratio of garlic oleoresin, turmeric oleoresin, capsicum oleoresin, rosemary extract, and wild oregano essential oils), (iv) 3% (wt/wt) OA blend (1:1 ratio of lactic, propionic, formic, and benzoic acids), (v) 2% (wt/wt) MCFA blend (1:1 ratio of caproic, caprylic, and capric acids), and (vi) 1% sodium bisulfate (Jones-Hamilton Co, Walbridge, OH). The four matrices included (i) feather meal, (ii) avian blood meal, (iii) porcine meat and bone meal, and (iv) poultry by-product meal. Matrices had not been previously treated with other chemicals. One kilogram of each feed matrix was placed in a laboratory-scale ribbon mixer, in which the liquid chemicals were fogged into the feed and the dry powder treatment was mixed directly into the mixer.

Inoculum preparation. A total of 100 μl of *S. enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028) was placed into 10 ml of Trypticase soy broth (TSB; Difco, BD, Franklin Lakes, NJ) and grown for 24 h at 35°C. The culture was then centrifuged at 5,000 × *g*. Next, 7 ml of the TSB supernatant was removed. The remaining 3 ml of supernatant was vortexed to remove cells from the side of the tube and then was used for the inoculation.

Feed ingredient inoculation. Of each chemically treated matrix, 120 g was weighed and placed in plastic, for a total of 24 containers for inoculation. A pump spray nozzle was then used to disperse the cells across each matrix. The pump nozzle was first cleaned using ethanol, and then TSB was used to flush the pump. Following the cleaning step, the spray nozzle was placed into the 3 ml of *Salmonella* Typhimurium ATCC 14028 cells, which were then applied to the feed treatments. Once the inoculum was added, each container was shaken to mix the inoculum throughout the matrix. Each inoculated matrix was then stored in containers at room temperature throughout the 42-day experiment. On each analysis day, the containers were opened inside a hood to prevent outside contamination.

Microbiological analysis. On each analysis day, three samples were taken from each container. A total of 11 g per sample was placed into 99 ml of buffered peptone water and mixed. Samples were then diluted to 10^3, 10^2, and 10^1 and were plated on xylose lysine deoxycholate (XLD) agar, with a limit of detection of less than 100 CFU/g of feed matrix. Procedures were repeated on days 0, 1, 3, 7, 14, 21, and 42 to evaluate chemical effectiveness over time.

Statistical analysis. Data were analyzed using the GLIMMIX procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC) after log transformation; chemical treatment and feed matrix were fixed effects and day a repeated measure. There were three replicates of each chemical treatment × feed matrix combination at each sampling day. Differences were considered statistically significant at $P < 0.05$.

RESULTS

All main effects and interactions were highly significant ($P < 0.001$). Overall, the MCFA, commercial formaldehyde product, OA, and EO treatments each had a lower concentration of *Salmonella* Typhimurium ATCC 14028 compared with the control ($P < 0.05$). The MCFA treatment and commercial formaldehyde product were the most successful at preventing cross-contamination from *Salmonella* Typhimurium ATCC 14028 (0.51 and 0.65 CFU/g, respectively; Table 1); less successful were the OA treatment (1.20 CFU/g) and the EO treatment (2.10 CFU/g). The sodium bisulfate treatment was similar to the control ($P = 0.14$; 2.38 versus 2.56 CFU/g).

Differences were also observed when evaluating the main effect of feed matrix. Values for avian blood meal and porcine meat and bone meal were similar ($P = 0.36$; 1.73 and 1.82 CFU/g, respectively), but greater prevention of cross-contamination by *Salmonella* Typhimurium ATCC 14028 was seen in feather meal and poultry by-product meal ($P < 0.05$; 1.36 and 1.36 CFU/g).

Time also played a major role in the degradation of *Salmonella* Typhimurium ATCC 14028. Over the 42 days of the experiment, the quantity of *Salmonella* Typhimurium ATCC 14028 detected decreased linearly ($P < 0.05$; 4.50, 2.65, 1.75, 0.95, 0.49, 0.50, and 0.13 CFU/g for days 0, 1, 3, 7, 14, 21, and 42, respectively). With the exception of days 14 and 21 ($P = 0.93$), the quantity of *Salmonella* Typhimurium ATCC 14028 detected each day differed from other days ($P < 0.05$).

The MCFA mixture was the most effective chemical treatment in avian blood meal, feather meal, and meat and bone meal, followed by the commercial formaldehyde treatment. The commercial formaldehyde treatment and MCFA mixture were the chemicals most successful at reducing the quantity of *Salmonella* Typhimurium ATCC 14028 in poultry by-product meal ($P < 0.05$; Table 2).

In evaluations of efficacy over time, the MCFA and commercial formaldehyde treatments were the most effective at mitigating *Salmonella* Typhimurium ATCC 14028 during the entire experimental period ($P < 0.05$; Table 3), particularly over the days soon after treatment and inoculation. The OA treatment was also effective at mitigating *Salmonella* Typhimurium ATCC 14028 over the experimental period, but it required more time for effectiveness than the MCFA or commercial formaldehyde

TABLE 1. *Treatment main effects for chemically treated* Salmonella-*inoculated feed matrices*[a]

Day:								
0	1	3	7	14	21	42	SEM	*P*
4.50 A[b]	2.65 B	1.75 C	0.95 D	0.49 E	0.50 E	0.13 F	0.09118	<0.0001
Chemical treatment:								
Untreated positive control	Commercial formaldehyde	Essential oil	Medium chain fatty acids	Organic acid	Sodium bisulfate			
2.56 A	0.65 D	2.10 B	0.51 D	1.20 C	2.38 A		0.08442	<0.0001
Feed matrix:								
Avian blood meal	Feather meal	Porcine meat and bone meal	Poultry by-product meal					
1.73 A	1.36 B	1.82 A	1.36 B				0.06893	<0.0001

[a] Four feed matrices were treated with six different chemical treatments, inoculated with *Salmonella enterica* subsp. *enterica* serovar Typhimurium, and plated on XLD over 42 days. Values are presented in log CFU per gram.
[b] Values in rows not sharing the same letter are significantly different ($P \leq 0.05$).

treatments ($P < 0.05$). Interestingly, the EO and sodium bisulfate treatments were similar to the untreated control during the duration of the 42-day experiment.

Feed matrix had a significant impact on *Salmonella* Typhimurium ATCC 14028 concentration over the 42-day analysis period. The *Salmonella* Typhimurium ATCC 14028 concentration in feather meal was lower ($P < 0.05$) than in the other feed matrices on days 0 and 1 postinoculation. However, poultry by-product meal had a lower ($P < 0.05$) *Salmonella* Typhimurium ATCC 14028 concentration than the other matrices from 3 to 42 days after inoculation (Table 4). Interestingly, we observed that the blood meal and meat and bone meal still had residual levels of *Salmonella* Typhimurium ATCC 14028 by the end of the 42-day experimental period, whereas the blood meal and feather meal matrices self-mitigated over time.

DISCUSSION

The purpose of this proof-of-concept experiment was to evaluate whether categories of chemical treatments could prevent postprocessing *Salmonella* Typhimurium ATCC 14028 contamination, which was determined by quantifying the concentration of *Salmonella* Typhimurium ATCC 14028 colonies present by XLD plating. Surprisingly, the MCFA mixture performed similarly to the commercial formaldehyde product. The commercial formaldehyde product used in this experiment is intended to inhibit mold growth and has been shown to maintain feed and feed ingredients in *Salmonella*-negative status *(4)*. The product is used in the animal feed industry to prevent recontamination in the manufacturing, storage, and transportation of animal feed or feed ingredients *(4)*. Meanwhile, MCFA, such as capric and caprylic acid, have been shown to be effective against *E. coli* and *Salmonella* spp. growth *(17)*. Caprylic acid added to feed has been shown to decrease the quantity of *Salmonella* spp. colonization in broiler chicks *(15)*. Although the added concentrations of MCFA in that experiment were 0.7 and 1%, the concentration in our experiment was nearly double that because we were testing a proof-of-concept to first assess whether an extremely high combination of chemicals in a single chemical category was effective in preventing *Salmonella* Typhimurium ATCC 14028 cross-contamination. We wholly recognize that our tested levels are not realistic inclusion levels for animal feed, but these results provide a direction for future research emphasis. According to our findings, more research is warranted to identify the mode of action of MCFA in preventing cross-contamination of *Salmonella* Typhimurium ATCC 14028 in animal feed, as well as to elucidate the effectiveness of lower doses and of single MCFA inclusion levels.

TABLE 2. *Chemical × feed matrix interaction for chemically treated* Salmonella-*inoculated feed matrices*[a]

Item	*Salmonella* +	Commercial formaldehyde	Essential oil	Medium chain fatty acids	Organic acid	Sodium bisulfate	SEM	*P*
Blood meal	3.28 A[b]	0.72 IJ	1.36 GH	0.54 IJK	1.54 FGH	2.91 AB	0.1688	<0.0001
Feather meal	2.68 BC	0.32 J	2.09 DE	0.21 K	0.47 IJK	2.40 CD		
Meat/bone meal	2.38 CD	0.82 I	3.19 A	0.54 IJK	1.49 FGH	2.46 BCD		
Poultry by-product	1.90 EF	0.73 IJ	1.75 EFGH	0.73 IJ	1.30 H	1.77 EFG		

[a] Four feed matrices were treated with six different chemical treatments, inoculated with *Salmonella enterica* subsp. *enterica* serovar Typhimurium, and plated on XLD over 42 days. Values are presented in log CFU per gram.
[b] Values in columns not sharing the same letter are significantly different ($P \leq 0.05$).

TABLE 3. *Chemical × time interaction for chemically treated* Salmonella-*inoculated feed matrices*[a]

Item	Day: 0	1	3	7	14	21	42	SEM	*P*
Salmonella +	5.45 A[b]	4.55 C	3.12 EF	2.42 GH	1.02 JK	1.19 JK	0.19 LM	0.2234	<0.0001
Commercial form	3.57 E	0.33 LM	UND M	UND M	0.26 LM	0.37 LM	UND M		
Essential oils	5.22 AB	3.71 DE	2.88 FG	1.45 IJ	0.71 KL	0.36 LM	0.36 LM		
Organic acids	4.64 BC	2.44 GH	1.14 JK	UND M	0.17 LM	UND M	UND M		
Medium chain fatty acids	2.35 GH	0.66 KL	0.17LM	UND M	UND M	0.36 LM	UND M		
Sodium bisulfate	5.75 A	4.21 CD	3.16 EF	1.85 HI	0.77 KL	0.72 KL	0.23 LM		

[a] Four feed matrices were treated with six different chemical treatments, inoculated with *Salmonella enterica* subsp. *enterica* serovar Typhimurium, and plated on XLD over 42 days. Values are presented in log CFU per gram. UND, undetectable (counts that averaged less than 100 CFU/g).

[b] Values in columns not sharing the same letter are significantly different ($P \leq 0.05$).

This research confirmed that MCFA were more antibacterial than OA, a concept that has been previously reported *(25)*. Although less effective than MCFA or formaldehyde treatment, the inclusion of the OA blend in rendered ingredients was still effective in preventing *Salmonella* Typhimurium ATCC 14028 postprocessing contamination compared with the control. Previous research supports the bactericidal activity of OA. Propionic acid has been shown to destroy 90% of the cell population within 1 h, and formic acid within 3 h, of treatment *(7)*. A blend of propionic and formic acids was evaluated; it performed similarly to the OA treatment in this study *(7)* and was previously reported to be less successful than a formaldehyde control *(6)*. The proposed mode of action of OA treatment to mitigate *Salmonella* Typhimurium ATCC 14028 contamination suggests that OA penetrate the cell membrane and enter the bacterial cell's cytoplasm; there they dissociate, causing the pH of the cell to decrease and the cell to atrophy *(5)*. There are further advantages to OA treatment compared with formaldehyde: OA is thought to be relatively stable in feed and can occur naturally in living organisms and, therefore, may have greater consumer appeal when listed on an ingredient label *(26)*.

EO are also consumer-friendly chemical additives that were effective in decreasing the risk of *Salmonella* Typhimurium ATCC 14028 cross-contamination compared with the control. Previous research supports our findings that EO effectively mitigate *Salmonella* Typhimurium ATCC 14028. Garlic and oregano have been shown to be effective at mitigating *Salmonella* spp.; they have MICs of 729 and 417 ppm and maximal tolerated concentrations of 52 and 104 ppm, respectively *(10)*. Rosemary has also been shown to be effective against *Salmonella* spp. contamination, with a MIC of 0.3 % (vol/vol) and minimal bactericidal concentration of 0.5% (vol/vol) against *E. coli* contamination *(26)*. The phenolic compounds in EO are thought to be essential to their mode of action as bactericidal compounds *(14)*. Some EO contain phenol compounds that are thought to interact with and disrupt the cell membranes of bacteria, causing the cells to lose functional properties and leak the inner cell materials *(14)*. The EO treatment in this study was effective, but not to the same magnitude as MCFA, formaldehyde, or OA inclusion. Still, its effectiveness was demonstrated compared with the control and may vary within different targeted ingredients.

The sodium bisulfate treatment was evaluated due to its commercial availability in the pet food and poultry industries. The chemical additive, which has acidulate and desiccant properties, is in a granular form that makes it attractive for use within dry bulk manufacturing systems, such as animal feed mills *(23)*. However, the addition of the product did not prevent *Salmonella* Typhimurium ATCC 14028 postprocessing contamination of the tested ingredients compared with the control. Potentially, this dry powder form was partially responsible for the product's lack of mitigation properties observed in this experiment; because the granular form does not as easily coat ingredient particles, the likelihood of the product contacting *Salmonella* Typhimurium ATCC 14028 cells is reduced. Use of a smaller particle size or of a liquid form of the product might

TABLE 4. *Feed matrix × time interaction for chemically treated* Salmonella-*inoculated feed matrices*[a]

Item	Day: 0	1	3	7	14	21	42	SEM	*P*
Blood meal	4.85 A[b]	2.85 C	1.87 EF	1.27 GH	0.52 JKL	0.60 IJKL	0.13 LM	0.1824	<0.0001
Feather meal	3.41 B	2.12 E	1.58 FG	1.06 HI	0.48 KLM	0.86 HIJK	UND M		
Meat/bone meal	4.86 A	2.77 CD	2.31 DE	1.00 HIJ	0.84 HIJK	0.53 JKL	0.39 KLM		
Poultry by-product	4.87 A	2.86 C	1.22 GH	0.48 KLM	0.11 LM	UND M	UND M		

[a] Four feed matrices were treated with six different chemical treatments, inoculated with *Salmonella enterica* subsp. *enterica* serovar Typhimurium, and plated on XLD over 42 days. Values are represented by log CFU per gram. UND, undetectable (counts that averaged less than 100 CFU/g).

[b] Values in columns not sharing the same letter are significantly different ($P \leq 0.05$).

have led to more successful mitigation. This concept could apply to all solid-phase mitigants, suggesting that liquid- or gaseous-phase chemical additives may be more effective in *Salmonella* Typhimurium ATCC 14028 mitigation due to their improved coating characteristics.

Time, MCFA, commercial formaldehyde product, OA, and EO all decreased the presence of *Salmonella* Typhimurium ATCC 14028 in feed ingredients, but those results can vary based on the feed ingredient. *Salmonella* Typhimurium ATCC 14028 concentration was relatively stable in avian blood meal over 42 days, compared with 21 days in the other three feed ingredients. The MCFA and formaldehyde treatments were most effective at preventing postprocessing contamination of rendered protein meals. Further research is needed to evaluate the effectiveness of MCFA inclusion at more practical inclusion levels.

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