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Research Note

Evaluation of Liquid and Dry Chemical Treatments To Reduce *Salmonella* Typhimurium Contamination on Animal Food Manufacturing Surfaces

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ABSTRACT

Salmonella can be isolated from animal food, ingredients, and animal food manufacturing surfaces. There are limited data regarding the sanitation of animal food manufacturing surfaces. This experiment evaluated the effects of nine chemical treatments on reduction of *Salmonella* Typhimurium contamination on various manufacturing surfaces. This experiment was a 9 $\times$ 5 factorial with nine chemical treatments and five surfaces. The nine chemical treatments included one with no inoculation or sanitation treatment (negative control). In the other eight treatments, inoculation with *Salmonella* Typhimurium was followed by either no sanitation treatment (positive control) or treatment with ground corn; liquid commercial formaldehyde; liquid food-grade sanitizer; liquid medium chain fatty acid blend of caprylic, caproic, and capric acids (MCFA); dry commercial calcium propionate; dry commercial acidulant; and dry commercial benzoic acid. The five surfaces included stainless steel, plastic, polypropylene tote bag, rubber belt, and rubber tire. Plastic had higher levels of *Salmonella* in the positive control than did the polypropylene tote bag; other surfaces had intermediate levels ($P < 0.05$). Surfaces treated with formaldehyde had no detectable *Salmonella* after treatment, and surfaces treated with MCFA had at least a 4-log reduction compared to the control ($P < 0.05$). The dry acidulant was the most effective dry sanitizer tested, but it had no impact on *Salmonella* concentration on rubber tires ($P < 0.05$). Whereas liquid sanitizers were the most effective in this experiment, they have limitations for use in dry bulk systems. In summary, formaldehyde, food-grade sanitizer, and MCFA were the most effective chemical treatments to reduce *Salmonella* surface contamination. Surface type can also influence *Salmonella* mitigation strategies; specifically, stainless steel and plastic can be more challenging to sanitize within animal food facilities.

HIGHLIGHTS

- The efficacy of sanitizers can be impacted by surface type.
- Liquids were the most effective chemical treatments to reduce *Salmonella*.
- The dry acidulant was the most effective dry sanitizer tested.

Key words: Decontamination; Feed safety; *Salmonella*

Globally, salmonellosis affects over one million people, with 380 human deaths each year in the United States *(2)*. Recent changes in the farm-to-fork initiative by the US Food and Drug Administration have led animal food manufacturing facilities to place a greater emphasis on the control of biological hazards, such as *Salmonella (21)*. It has been demonstrated that *Salmonella* and other pathogens may potentially be introduced into facilities through ingredients and by employees *(7, 14, 16)*. Thermal processing, such as extrusion or pelleting, can eliminate or reduce biological hazards in animal food *(5, 17)*. However, postprocessing cross-contamination can occur during the manufacturing, storage, and transportation of the finished product; dust, air, or employee handling can cause residual contamination in finished product processing areas *(12)*. Sanitization of postprocessing surfaces is one way to prevent postprocessing cross-contamination. However, there are few data to evaluate the efficacy of various sanitizers on animal food manufacturing surfaces *(9, 16)*. Available data are extrapolated from the literature about human food manufacturing and tend to be focused on liquid sanitizers. Whereas liquid sanitation has been shown to be effective against biological hazards, including biofilm-forming bacteria, its use is challenging in a traditional animal food manufacturing facility, which is typically a dry bulk system not designed with clean-in-place equipment *(3, 20)*. There are a variety of surfaces within these facilities, and there are no published data that evaluate the efficacy of sanitizers for use in animal food manufacturing facilities on various surface types. Therefore, the objective of this experiment was to identify successful sanitizing treatments

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to remove *Salmonella* Typhimurium from a variety of common animal food manufacturing surfaces.

MATERIALS AND METHODS

Inoculum and surface preparation. *Salmonella* Typhimurium (ATCC 14028) was stored at –80°C and was inoculated into 10 mL of trypticase soy broth (TSB; Difco, BD, Franklin Lakes, NJ) and incubated for 24 h at 37°C. Next, samples were streak plated onto tryptic soy agar (Difco, BD) plates held at 35 ± 2°C for 24 ± 2 h. Single colonies were then used to inoculate TSB and were incubated at 35 ± 2°C for 24 ± 2 h. Next, 1 mL of *Salmonella* inoculum broth was pipetted onto sterile coupon surfaces and spread using a cell spreader, as described by Bowman et al. *(1)*. Surface coupons represented new surfaces common in manufacturing environments: stainless steel (to represent equipment surfaces) (stainless steel 316, Built-So-Well, Manhattan, KS), a plastic bucket from a bucket elevator conveyor (Dura Bucket National Oats Co., Collinsville, IL), a rubber belt from a bucket elevator conveyor (Maxi-Lift Inc., Addison, TX), a rubber tire (Firestone Tire and Rubber Company LLC, Nashville, TN), and woven polypropylene from a tote bag commonly used to store and transport animal food (The MegaSack Corp., Magnolia, AR). Coupons (103.23-cm^2 squares) were placed in sterile petri dishes.

Surface treatment. Coupons were incubated at 35 ± 2°C for 24 ± 2 h to allow biofilm formation of *Salmonella* Typhimurium. Next, 1 mL of liquid or 15 g of dry treatment was spread onto each surface for 15 min to allow for complete surface coverage. Immediately after dry treatment, sterile forceps were used to remove excess material and the coupon was gently tapped twice. There were nine treatments, the first with no inoculation or sanitation treatment (negative control). In the other eight treatments, inoculation with *Salmonella* Typhimurium was followed by either no sanitation treatment (positive control) or treatment with ground corn; liquid 30% formaldehyde-based commercial product (Sal CURB, Kemin Inc., Des Moines, IA); liquid 0.03% ammonium chloride, 10.89% isopropanol, and 0.045% hydrogen peroxide–based commercial food-grade sanitizer (DrySan Duo, Ecolab, St. Paul, MN); proprietary blend of liquid medium chain fatty acid blend of caprylic, caproic, and capric acids described by Cochrane et al. (MCFA) *(4)*; dry commercial 97% calcium propionate (SHIELD CA, Kemin Inc.); dry commercial acidulant 91.5% sodium bisulfate (Jones-Hamilton Co., Walbridge, OH); and dry commercial 99.9% benzoic acid (VevoVitall, DSM Nutritional Products Inc., Parsipanny, NJ). Chemical treatments were grouped by dry and liquid treatments. Dry treatments included SHIELD CA, SBS, and Vevo Vitall; liquid treatments included Sal CURB, DrySan Duo, and MCFA.

Sample plating and enumeration. After the residue of chemical treatment was removed, coupons were swabbed (PUR-Blue swab sampler with 5 mL of neutralizing buffer, large tip swab; World Bioproducts LLC, Woodinville, WA) as described by Davidson et al. *(6)* and were vortexed prior to dilution *(1, 6)*. Samples were then serial diluted (10^{-1} to 10^{-6}) in neutralizing broth (EMD Chemicals, Darmstadt, Germany) and spread to TSA plates. Plates were incubated at 35 ± 2°C for 24 ± 2 h and then enumerated.

Statistical analysis. Data were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) as a completely randomized design with the main effects of surface and treatment, the interaction of treatment × surface, and a preplanned contrast of dry versus wet chemical treatments. There were three replicates per treatment. All results were log transformed and presented as *Salmonella* CFU/cm^2. Differences were considered statistically significant at $P < 0.05$ and marginally significant at $P < 0.10$.

RESULTS AND DISCUSSION

Salmonella mitigation strategies for animal food manufacturing surfaces should include minimization of entry, point-in-time mitigation, and prevention of postprocessing cross-contamination. One strategy is to ensure that postprocessing equipment surfaces are not contaminated with biological hazards. Dust collected from the animal food manufacturing surface is likely the culprit of this contamination. Removal of dust may not remove biological hazards, particularly bacterial hazards. Physical cleaning has been shown to be ineffective at reducing concentrations of *Enterococcus faecium* on equipment in animal food manufacturing facilities *(10)*. Huss et al. found that highly intensive liquid sanitation and heat were required to completely remove biological hazards from an animal food manufacturing facility *(10)*. Thus, sometimes animal food manufacturing equipment may require substantial sanitization: sanitation of surfaces can reduce cross-contamination and equipment throughout the facility may be decontaminated if an undesirable microorganism has been established. Use of liquid sanitizers typically requires physical cleaning, chemical treatment, rinsing with water, and complete drying. Because these activities are typically not practical for animal food manufacturing facilities, use of dry sanitizers may be more practical if they are found to be effective.

All main effects and interactions were highly significant ($P \leq 0.001$). Commercial formaldehyde, commercial food-grade sanitizer, MCFA, and dry commercial acidulant reduced *Salmonella* concentrations compared to the positive control ($P < 0.05$, Table 1). The most effective treatment was commercial formaldehyde; direct application of the commercial product containing 30% formaldehyde resulted in no detectable *Salmonella* on all tested surfaces (6.7 CFU/cm^2 mean reduction; $P < 0.05$). The other liquid sanitizers also reduced ($P < 0.05$) *Salmonella* concentrations on surfaces compared to the positive control; MCFA resulted in a 5.8-log mean reduction and the ready-to-use liquid commercial food-grade sanitizer in a 2.9-log reduction compared to the positive control. Previous research found that treatment of animal foods with formaldehyde and MCFA prior to inoculation with *Salmonella* was highly effective in prevention of cross-contamination *(4)*. To demonstrate that dry sanitation was effective beyond physical action, a treatment of dry corn without chemical was tested and yielded no difference compared to the positive control (6.1 versus 6.7 CFU/cm^2; $P < 0.05$). The dry commercial acidulant was the only dry treatment that reduced ($P < 0.05$) the *Salmonella* concentration below the positive control level, with a 1.3-log reduction. Liquid sanitation treatments, including commercial formaldehyde, medium chain fatty acid blend, and food-grade sanitizer,

TABLE 1. *The mean* Salmonella *counts on manufacturing surfaces after various chemical treatments*[a]

Parameter	Value[b]
Salmonella (log/cm^2)	Mean count
Surface	
Plastic	4.2 A
Polyethylene tote bag	3.5 BC
Rubber belt	3.3 C
Stainless steel	4.5 A
Rubber tire	4.0 AB
Treatment	
Negative control	NG E
Positive control	6.7 A
Untreated ground corn	6.1 AB
Concentrated liquid commercial formaldehyde[c]	NG E
Ready-to-use liquid commercial food-grade sanitizer[d]	3.8 C
Concentrated liquid medium chain fatty acid blend[e]	0.9 D
Concentrated dry commercial calcium propionate[f]	6.0 AB
Concentrated dry commercial acidulant[g]	5.4 B
Concentrated dry commercial benzoic acid[h]	6.2 A
P	
Surface	0.001
Treatment	<0.0001
Surface × treatment	0.001
Dry vs liquid treatment	<0.0001
SEM	
Surface	0.19
Treatment	0.26
Surface × treatment	0.58

[a] This experiment was conducted in a 5 × 9 factorial with three replicates per treatment. NG, no growth of *Salmonella* detected after 24 h of incubation.
[b] Means followed by different letters differ ($P < 0.05$).
[c] Commercial 30% formaldehyde (Sal CURB, Kemin Inc.).
[d] Commercial 0.03% ammonium, 10.89% chloride isopropanol, and 0.045% hydrogen peroxide sanitizer (DrySan Duo, Ecolab).
[e] Medium chain fatty acid blend (1:1:1, caprylic, caproic, and capric acids) *(3–5)*.
[f] Commercial 97% calcium propionate (SHIELD CA, Kemin Inc.).
[g] Commercial acidulant (sodium bisulfate, Jones-Hamilton Co.).
[h] Commercial 99.9% benzoic acid (VevoVitall, DSM Nutritional Products Inc.).

were more effective than dry treatments (2.9 to 6.7 CFU/cm^2 log reduction; $P < 0.0001$).

Surface type also impacted *Salmonella* concentration ($P = 0.001$); plastic and stainless steel had greater mean *Salmonella* concentrations in the positive controls compared to rubber tire, rubber belt, and polypropylene tote bag (Table 1, $P < 0.05$; 4.2 and 4.5 versus 4.0, 3.5, and 3.3 CFU/cm^2). Previous research has shown that Buna-n-rubber and polyethylene coating have bacteriostatic and hydrophobic actions toward *Salmonella, Listeria monocytogenes,* and protein substrates *(13, 19)*.

Effect of chemical treatment on plastic surfaces. Plastic surfaces in animal food manufacturing facilities are common in bucket elevator conveyors and utensils, such as shovels. Plastic elevator buckets, used as the source of plastic in this study, raise concern due to accumulation of organic material in the boot pit, or bottom, of bucket elevators, where they may harbor biological hazards. These plastic surfaces typically are smooth when new, as in the coupon sampled, but as they become gouged during normal equipment wear they may provide additional harborage for undesirable microorganisms. The initial *Salmonella* concentrations on plastic surfaces were among the highest on all surfaces tested and were significantly higher than on polyethylene tote bag (7.4 versus 5.8 log CFU/cm^2; $P < 0.05$; Table 2). Other surfaces had intermediate *Salmonella* concentrations on the positive control samples. Liquid commercial formaldehyde, food-grade sanitizer, MCFA, and the dry commercial acidulant reduced *Salmonella* concentrations on plastic compared to the positive control ($P < 0.05$; 7.4, 6.0, 5.8, and 1.0 CFU/cm^2 log reduction, respectively). This is promising because most dry sanitizers aim to achieve a 1-log or 90% reduction in *Salmonella (15)*. These dry acids are typically less effective than liquids due to the potential formation of biofilms, but they have greater consumer appeal and are more practical to implement compared to their liquid counterparts *(11)*. Because sodium bisulfate is generally recognized as safe and can be used as an animal food ingredient, it is practical for use in animal food manufacturing. The weak acid salt dissociates to have a two-phase action: it lowers the pH to limit bacterial growth and it desiccates the cytoplasm for a bactericidal effect that is effective in human foods *(11)*. Applied as a coating to pet food kibble, the product has been shown to reduce *Salmonella* contamination and reduce cross-contamination; thus, it has promising results as a sanitizer *(11)*. Treatment with dry calcium propionate and dry benzoic acid showed no significant reduction in *Salmonella* ($P > 0.05$) on plastic surfaces compared to the positive control.

Effect of chemical treatment on polypropylene tote bags. Biological hazards may be introduced when animal food manufacturing facilities reuse bags to store or transport animal food or when transportation bags are moved between farms and the facility. The reuse of tote bags is not recommended without proper cleaning, chemical sanitizing, and complete drying. However, this process is rarely completed by animal food manufacturers. Thus, residual material or dust on a bag, as well as any potential biofilms, may lead to harborage of undesirable microorganisms.

Salmonella contamination on polypropylene tote bags, which are commonly used to store and transport animal food, was the lowest among all tested surfaces and was significantly lower than on plastic surfaces ($P < 0.05$; 5.8 versus 7.4 CFU/cm^2, Table 2). Notably, these bags contain woven polypropylene plastic, which made the inoculation, sanitizer treatment, and swabbing more challenging. However, both formaldehyde and MCFA reduced surface contamination of *Salmonella* compared to the positive control ($P < 0.05$; 5.8 and 5.4 CFU/cm^2 log reduction). No

TABLE 2. *Effect of chemical treatment × surface interaction on* Salmonella *inoculated feed manufacturing surfaces*[a]

Surface	Plastic	Polyethylene tote bag	Rubber	Stainless steel	Tire
Salmonella (log/cm^2)					
Negative control	NG	NG	NG	NG	NG
Positive control	7.4 A	5.8 BCDEFGHI	6.1 ABCDEFGH	7.4 AB	6.6 ABCDEF
Untreated ground corn	7.3 ABC	5.2 GHI	4.9 HI	7.5A	5.5 DEFGHI
Concentrated liquid commercial formaldehyde[b]	NG	NG	NG	NG	NG
Ready-to-use liquid commercial food-grade sanitizer[c]	1.4 KL	4.0 IJ	2.9 KJ	5.3 FGHI	5.3 FGHI
Concentrated liquid medium chain fatty acid blend[d]	1.6 KL	1.4 KL	NG2	0.8	0.5
Concentrated dry commercial calcium propionate[e]	7.0 ABCD	5.2 FGH	5.3 EFGHI	7.0 ABCDE	5.7 CDEFGH
Concentrated dry commercial acidulant[f]	6.4 BCDEFGH	4.7 HI	5.2 FGHI	4.7 HI	6.8 ABCDEF
Concentrated dry commercial benzoic acid[g]	7.5 A	5.1 GHI	5.1 GHI	7.6 A	5.5 DEFGHI
P	0.001				
SEM	0.82				

[a] This experiment was conducted in a 5 × 9 factorial with three replicates per treatment. NG, no growth of Salmonella detected after 24 h of incubation. Means followed by different letters differ ($P < 0.05$).
[b] Commercial 30% formaldehyde (Sal CURB, Kemin Inc.).
[c] Commercial 0.03% ammonium, 10.89% chloride isopropanol, and 0.045% hydrogen peroxide sanitizer (DrySan Duo, Ecolab).
[d] Medium chain fatty acid blend (1:1:1, caprylic, caproic, and capric acids) *(3–5)*.
[e] Commercial 97% calcium propionate (SHIELD CA, Kemin Inc.).
[f] Commercial acidulant (sodium bisulfate; Jones-Hamilton Co.).
[g] Commercial 99.9% benzoic acid (VevoVitall, DSM Nutritional Products Inc.).

other treatment reduced *Salmonella* concentration on polyethylene tote bags compared to the control ($P > 0.05$).

Effect of chemical treatment on rubber belt surfaces. Similar to plastic surfaces, rubber presents a specific concern because it is used on bucket elevators. Rubber belts in these conveyors typically become cracked and pitted, forming additional surfaces to harbor biological hazards. Previous research has demonstrated that rubber surfaces are already more difficult to sanitize to prevent increased growth of certain bacteria, including *Listeria monocytogenes*; however, those surfaces were less bacteriostatic with respect to *Salmonella* Typhimurium *(19)*. After rubber surfaces were treated with commercial formaldehyde and MCFA, there was no detectable growth of *Salmonella* ($P < 0.05$; 6.1 CFU/cm^2 log reduction versus positive control). Treatment with commercial food-grade sanitizer also effectively reduced *Salmonella* on rubber compared to the positive control ($P < 0.05$; 3.2 CFU/cm^2 log reduction). All dry treatments resulted in levels of *Salmonella* similar to the control ($P > 0.05$).

Effect of chemical treatment on stainless steel surfaces. One of the most common surfaces within animal food manufacturing facilities is stainless steel. Whereas its positive control had one of the highest concentrations of *Salmonella* of all tested surfaces, it was statistically similar to all other surfaces tested ($P > 0.05$). The most effective sanitizer on stainless steel was commercial formaldehyde, followed by MCFA, commercial dry acidulant, and the commercial food-grade sanitizer ($P < 0.05$; 7.4, 6.6, 2.7, and 1.9 CFU/cm^2 log reduction, respectively, compared to the positive control). The commercial calcium propionate and benzoic acid sanitizers were not effective at reducing *Salmonella* concentration compared to the control ($P > 0.05$). Møretrø et al. *(16)* demonstrated that the most effective chemical treatment to reduce *Salmonella* Senftenberg 1702-1 and *Salmonella* Agona 71-3 dried onto stainless steel surfaces in animal food manufacturing facilities was 70% ethanol (>4-log reduction), in comparison with commercial acids, aldehyde, peroxygens, and chloride products. A common chemical treatment in laboratory settings, 70% ethanol is highly effective; however, it is impractical to implement on a large scale in animal food manufacturing facilities because residues require rinsing with water and complete drying prior to resumption of manufacturing *(16)*.

Effect of chemical treatment on tires. Although not part of the traditional animal food manufacturing environment, vehicle tires frequently enter animal food manufacturing facilities and drive over exposed ingredient pits while animal food is unloaded. Tire contamination may lead to cross-contamination of other surfaces or animal food. Some facilities have taken steps to sanitize vehicle tires before they enter facilities to limit their impact as a potential vector. Again, the most effective sanitizing treatment to remove *Salmonella* contamination included commercial formaldehyde and MCFA ($P < 0.05$; 6.6 and 6.1 CFU/cm^2 log reduction, respectively). No other sanitizer reduced *Salmonella* contamination compared to the control ($P > 0.05$).

Prior to applying a sanitizer treatment to any surface, cleaning is necessary to reduce surface tension and remove organic material. Effective cleaning, which may require both physical cleaning and the use of cleaning solutions, removes biofilm formations and allows for subsequent penetration and removal of vegetative bacteria by a sanitizer. Inadequate removal of organic matter during physical cleaning can provide adequate conditions for bacterial growth, increase cross-contamination, and reduce sanitizer efficacy. Organic material removal can be challenging for animal food facilities due to dust formation during production. Dust has been shown to cross-contaminate surfaces with porcine epidemic diarrhea virus and *Enterococcus faecium (8, 10)*. To control *Salmonella* within a facility, microbial growth requirements must be considered, including water activity, pH, and temperature range *(18)*. Thermal mitigation, reducing water activity, and acidifying (usually with a sanitizer) can reduce *Salmonella* contamination by creating unfavorable conditions for microbial growth.

Evaluation and selection of a sanitizer should consider microbial efficacy, practicality of application, application time, impact of surface type on effectiveness and corrosiveness, and cost *(15)*. Several sanitizers used in this study are highly corrosive and can cause metal pitting and degradation, creating additional niches for bacterial harborage. Sanitizer corrosiveness was not measured in this experiment; however, it is an important aspect to consider when evaluating sanitizers. Additional research is warranted to consider the use of a quaternary ammonium compound sanitizer and should include measures of equipment corrosiveness.

In summary, animal food manufacturing surfaces may be highly contaminated with *Salmonella* Typhimurium, and plastic surfaces are more likely to be contaminated than polyethylene tote bags. The physical action of unground corn without chemical treatment did not reduce *Salmonella* Typhimurium concentration on animal food manufacturing surfaces. Concentrated commercial formaldehyde was highly effective at reducing *Salmonella* contamination to undetectable levels on all tested surfaces. Treatments with medium chain fatty acid blend and commercial food-grade sanitizer were also effective at reducing *Salmonella* contamination on most surfaces. The dry commercial acidulant reduced the *Salmonella* concentration on most surfaces by approximately 1 log and was, thus, the most effective dry product tested. The use of a commercial dry calcium propionate product or a commercial dry benzoic acid product did not impact *Salmonella* concentration of surfaces compared to the positive control.

Data from this study are valuable as a starting point to identify potentially effective sanitizers, but additional research is warranted to determine practical dosages and application methods of liquid sanitizers on animal food manufacturing surfaces in an industry setting. Furthermore, more research is needed to identify or develop highly effective dry sanitizers that are able to penetrate or remove biofilms while preserving equipment integrity.

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