Research Note: Evaluating the roles of surface sanitation and feed sequencing on mitigating *Salmonella* Enteritidis contamination on animal food manufacturing equipment

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ABSTRACT The objective of this study was to evaluate the efficacy of flushing surfaces with untreated feed vs. the use of 2 different dry chemical sanitizers on residual surface and feed *Salmonella* Enteritidis contamination. First, a *Salmonella*-negative batch of poultry feed was mixed in 9 laboratory-scale paddle mixers. A feed sample was collected, and targeted locations on surfaces within the mixer were swabbed to confirm *Salmonella*-negative. Next, a *Salmonella*-positive batch of poultry feed was mixed, sampled, and mixer surfaces swabbed. Mean *Salmonella* Enteritidis contamination across all 9 mixers were 3.63 cfu/g for sampled feed and 1.27 cfu/cm^2 for surface contamination. Next, the mixers manufactured one of the following treatments (3 mixers/treatment): 1) none (control); 2) a commercially available essential oil blend; or 3) rice hulls treated with a 10% concentration of a propriety blend of medium-chain fatty acids (**MCFA**). After each treatment, each mixer manufactured another 2 batches of *Salmonella*-free feed (sequence 1 and sequence 2). Feed samples were collected, and surfaces were swabbed between each batch of feed. Manufacturing sequence ($P < 0.0001$) but not treatment ($P > 0.05$) impacted feed or surface contamination of *Salmonella* Enteritidis. There was *Salmonella*-positive residue in the batch of feed manufactured immediately after the positive control batch. However, no *Salmonella* residue was detected in batches of feed treated with either the commercial essential oil blend or MCFA. Low levels of *Salmonella* residue were observed from either feed (0.7 cfu/g for commercial essential oil blend) or surfaces (0.1 cfu/cm^2 for MCFA) manufactured in sequence 1, but no residue was observed in sequence 2. These data suggest that sequencing of feed during manufacturing reduces *Salmonella*-positive contamination within animal food and on manufacturing surfaces, particularly after the second batch or with the use of chemical treatments.

Key words: animal food, feed manufacturing, *Salmonella* Enteritidis

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INTRODUCTION

Recent changes in regulation and customer requirements are placing new pressure on the sanitation expectations for animal food manufacturing facilities, particularly those for livestock. Previous methods of sanitation of animal food contact surfaces have relied on "sequencing," where diets are manufactured in a strategic sequence to limit carryover from high-risk ingredients to specific feeds, and "flushing," where a pulse of animal food is conveyed through the manufacturing system to "flush" hazards through the manufacturing system. Although this method is successful for reducing the risk of chemical hazard carryover, there is limited research that evaluates if the same methods are effective at removing biological hazards from feed manufacturing surfaces, particularly those that form biofilms resistant to physical cleaning.

With higher emphasis on animal food safety extended to livestock species, feed mills will now need to reevaluate hazards within their facility to determine if hazard control is necessary. Most facilities will deem *Salmonella* spp. not requiring such control owing to a combination of low severity and probability in animal food. However, *Salmonella* Enteritidis is known to be potentially pathogenic to poultry, and the serotype is the 11th most frequent serotype found in animal food (Li et al., 2012; FDA, 2013). Thus, some poultry feed manufacturers may determine the control of *Salmonella* Enteritidis is necessary to prevent animal food from serving as a potential vector of the hazard.

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Methods to control biological hazards include Current Good Manufacturing Practices, Process Controls, Supply-Chain-Applied Controls, or Sanitation Controls. Sanitation Controls are appropriate in cases where an animal food manufacturing facility has concerns with undesirable microorganisms that may contaminate feed through cross contamination from manufacturing surfaces. While a great quantity of data have been generated regarding the efficacy of sanitizers in human food manufacturing facilities, very little data exist to evaluate the efficacy of sanitizers with animal food. Therefore, the objective of this experiment was to evaluate the efficacy of flushing surfaces with untreated feed vs. the use of 2 different dry chemical sanitizers on residual surface and feed *Salmonella* Enteritidis contamination.

MATERIAL AND METHODS

This study was conducted in the Biosafety Level 2 Cargill Feed Safety Research Center (FSRC) at Kansas State University. Procedures were approved by the Kansas State University Institutional Biosafety Committee #1058.

Preparation of Inoculum

Salmonella enterica subsp. Enterica Servar Enteritidis (ATCC 13076) was cultured, stored at −80°C, and inoculated to 10 mL of trypticases soy broth (Difco, Becton, Dickson and Company, Franklin Lakes, NJ) for 24 h at 37°C. Culture was further grown by transferring to fresh trypticases soy broth to produce 1 L of final inoculum with a concentration of 8.1 log cfu/mL.

Manufacturing of Salmonella-Negative Feed

A *Salmonella*-negative poultry diet was manufactured in the O.H. Kruse Feed Technology Innovation Center at Kansas State University in Manhattan, Kansas. Resulting feed was confirmed *Salmonella*-negative, subsampled into 2.2-kg batches, and stored in sealed packages at ambient conditions. *Salmonella*-free rice hulls were mixed with a 10% wt/wt addition of a proprietary medium-chain fatty acids (**MCFA**) 1:1:1 blend of caprylic, caproic, and capric acids described by Cochrane et al. (2016) and subsampled into 2.2-kg batches.

One batch of *Salmonella*-negative feed was mixed in each of 9 laboratory-scale mixers (Cabela's Heavy Duty Meat Mixer IK-541001; Cabela's Inc., Sidney, NE) for 5 min as the validated mix time. After mixing was complete, 2 samples of feed were collected from various locations within each mixer. Samples were stored at −20°C until analysis. Mixers were inverted to remove material but not physically cleaned, which resulted in a residue similar to that in commercial manufacturing conditions. Next, surfaces were then swabbed using a premoistened swab (PUR-Blue Swab Sampler with 5 mL of Neutralizing Buffer, Large Tip Swab; World Bioproducts LLC, Woodinville, WA) using procedures described by Bowman et al. (2015). Briefly, 4 various premeasured (103 cm^2) locations on the interior of the mixer, including 2 mixer sides, mixer paddles and shaft, and mixer lid were swabbed for surface contamination. Swabs were stored in collection containers at −20°C until analysis.

Manufacturing of Salmonella-Positive Feed

After manufacturing the Salmonella-free diets as aforementioned, the *Salmonella* Enteritidis broth inoculum was applied to 50 kg mash broiler chicken diet using a 100-kg paddle mixer (H.C. Davis Sons MFG Co. Inc., Bonner Springs, KS) with a pump sprayer, followed by 5 min of mixing. *Salmonella*-positive feed was discharged from the mixer and subsampled into 2.2-kg batches. These batches were then mixed in the 9 laboratory-scale mixers for 5 min, samples collected, mixers inverted, and surfaces swabbed using procedures described previously. Resulting *Salmonella*-positive feed contained 3.7 log cfu/g of *Salmonella* Enteritidis.

Chemical Flush and Sequencing

The 9 laboratory-scale mixers were then randomly assigned to 3 treatments with 3 mixers per treatment. Mixers were then subjected to one of the following treatments: 1) feed with no treatment (control); 2) feed mixed with concentrated commercial product containing a eubiotic blend of essential oils (CRINA; DSM Nutritional Products Inc., Parsippanny, NJ); or 3) rice hulls treated with MCFA. Treatment batches were mixed for 5 min, samples collected, mixers inverted, and surfaces swabbed using procedures described previously. Next, the 9 laboratory-scale mixers were used to manufacture 2 sequences of *Salmonella*-free feed (sequence 1 and sequence 2). Again, feed was mixed for 5 min, samples collected, mixers inverted, and surfaces swabbed using procedures described previously.

Sample Analysis

After collection of feed and surface swabs, samples were transported on ice to the microbiology laboratory for serial dilution, plated onto xylose deoxyribose agar, incubated, and enumerated for analysis of *Salmonella* in accordance with FDA BAM method (Feng et al., 2002). Below <10 cfu was determined below detectable limits.

Statistical Analysis

Data were log transformed and analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Inst. Ind., Cary, NC) as a completely randomized design with 3 replicates per treatment. Main effects included treatment (control vs. commercial essential oil blend vs. proprietary MCFA blend) and sequence nested within treatment (*Salmonella*-negative batch, *Salmonella*-positive batch, chemically treated batch, sequence 1, and sequence 2). *Salmonella* contamination in feed is

presented as *Salmonella* cfu/g, whereas contamination on surfaces is presented as cfu/cm^2. Differences were considered statistically significant at $P < 0.05$, and marginally significant at $P < 0.10$.

RESULTS AND DISCUSSION

This study evaluates 1 method to reduce the probability that animal food will be a vector for *Salmonella* entry into poultry farms and the human food chain. No samples of feed had detectable *Salmonella* after the *Salmonella*-negative batch of feed was manufactured (Table 1). One *Salmonella*-positive swab was collected from the lid of a mixer after the *Salmonella*-negative batch was manufactured. This was very low level contamination, and when averaged with swabs from 11 other swabs from that treatment, the mean level was lower than the 10 cfu/cm^2 detectable limit (Table 2).

All samples collected from laboratory-scale mixers after mixing the *Salmonella*-positive feed were confirmed to be *Salmonella*-positive, with an average contamination rate of 3.6 log cfu/g. This is a similar to the 3.7 log cfu/g *Salmonella* identified in feed after feed inoculation, and substantially lower than the 8.1 log cfu/mL *Salmonella* of the inoculum. We have previously reported a similar reduction of biological hazards or their surrogates from inoculum to feed (Huss et al., 2015; Cochrane et al., 2016). The contaminated feed increased the contamination of manufacturing surfaces, where 31 of the 36 swabs collected from manufacturing surfaces were positive for *Salmonella* after manufacturing the *Salmonella*-positive feed. These surfaces were contaminated with a lower quantity of *Salmonella* than the feed, with mean contamination of 1.3 cfu/cm^2 among the 36 samples. It is notable that surfaces had more than a 2-log reduction in *Salmonella* Enteritidis contamination compared with the level directly in the feed. However, this study effectively demonstrates that *Salmonella*-positive poultry feed can

Table 1. Impact of feed batch sequencing and chemical treatment on number of positive *Salmonella* Enteritidis feed samples and surface swabs.[1]

	Number of *Salmonella*-positive swabs/total swabs collected	
Treatment	Feed	Surfaces
Salmonella-negative batch	0/9	1/36
Salmonella-positive batch	9/9	31/36
Chemically-treated batch		
Control	—	—
Commercially available essential oil blend[2]	0/3	2/12
Rice hulls +10% medium chain fatty acid blend[3]	0/3	0/12
Sequence 1		
Control	1/3	4/12
Commercially available essential oil blend[2]	1/3	1/12
Rice hulls +10% medium chain fatty acid blend[3]	0/3	4/12
Sequence 2		
Control	0/3	0/12
Commercially available essential oil blend[2]	0/3	0/12
Rice hulls +10% medium chain fatty acid blend[3]	0/3	0/12

[1]*Salmonella*-negative feed was mixed in 9 laboratory-scale mixers, followed by *Salmonella*-positive feed (3.7 log cfu/g *Salmonella* Enteritidis), a chemically treated batch, and 2 *Salmonella*-negative feed sequences to evaluate traditional sequencing vs. 2 different chemical flushes on preventing batch-to-batch feed and manufacturing surface *Salmonella* contamination. Three treatments were tested: 1) no chemical (control); 2) a commercially available essential oil blend; or 3) rice hulls treated with a 10% concentration of a propriety blend of medium chain fatty acids. There were 3 mixers per treatment. One composite feed sample and 4 swabs of manufacturing surfaces were collected from each mixer after each batch and analyzed for *Salmonella* concentration. Detection limits were set at (<10 cfu/g or cfu/cm^2). Limits below the detection limit are designated as 0.

[2]CRINA (DSM Nutritional Products Inc., Parsippanny, NJ).

[3]10% wt/wt addition of a proprietary medium chain fatty acid 1:1:1 blend of caprylic, caproic, and capric acids described by Cochrane et al. (2015).

Table 2. Impact of feed batch sequencing and chemical treatment on level of *Salmonella* Enteritidis in feed samples and surface swabs.[1]

	Number of *Salmonella*-positive swabs/total swabs collected	
Sequence (treatment)	Feed	Surfaces
Salmonella-negative batch	0.0[b]	0.0[b]
Salmonella-positive batch	3.6[a]	1.3[a]
Chemically-treated batch		
Control	—	—
Commercially available essential oil blend[2]	0.0[b]	0.0[b]
Rice hulls + 10% medium chain fatty acid blend[3]	0.0[b]	0.0[b]
Sequence 1		
Control	0.8[b]	0.1[b]
Commercially available essential oil blend[2]	0.7[b]	0.0[b]
Rice hulls + 10% medium chain fatty acid blend[3]	0.0[b]	0.1[b]
Sequence 2		
Control	0.0[b]	0.0[b]
Commercially available essential oil blend[2]	0.0[b]	0.0[b]
Rice hulls + 10% medium chain fatty acid blend[3]	0.0[b]	0.0[b]
P =		
Treatment	0.194	0.259
Sequence (treatment)	<0.0001	<0.0001
SEM		
Treatment	0.43	0.23
Sequence (treatment)	0.29	0.11

[1]*Salmonella*-negative feed was mixed in 9 laboratory-scale mixers, followed by *Salmonella*-positive feed (3.7 log cfu/g *Salmonella* Enteritidis), a chemically treated batch, and 2 *Salmonella*-negative feed sequences to evaluate traditional sequencing vs. 2 different chemical flushes on preventing batch-to-batch feed and manufacturing surface *Salmonella* contamination. Three treatments were tested: 1) no chemical (control); 2) a commercially available essential oil blend; or 3) rice hulls treated with a 10% concentration of a propriety blend of medium chain fatty acids. There were 3 mixers per treatment. One composite feed sample and 4 swabs of manufacturing surfaces were collected from each mixer after each batch and analyzed for *Salmonella* concentration. Detection limits were set at (<10 cfu/g or cfu/cm^2). Limits below the detection limit are designated as 0.

[2]CRINA (DSM Nutritional Products Inc., Parsippanny, NJ).

[3]10% wt/wt addition of a proprietary medium chain fatty acid 1:1:1 blend of caprylic, caproic, and capric acids described by Cochrane et al. (2015).

[a,b]Different superscripts in the same column, $P < 0.05$.

contaminate animal food manufacturing surfaces and lead to carryover contamination in the next batch.

No feed was batched in the 3 mixers serving as the control for the chemically treated batch. The 3 mixers that were used to manufacture the commercially available essential oil blend had no residual contamination in feed samples. Two of the 12 surface samples had a low level of *Salmonella* residue, but the mean contamination among the samples was still lower than the 10 cfu/cm^2 detectable limit. No feed samples or surface swabs collected immediately after mixing the rice hulls treated with 10% proprietary blend of MCFA had detectable *Salmonella*.

After the chemically treated batch was manufactured, the laboratory-scale mixers were used to mix a *Salmonella*-negative diet as sequence 1. In the control mixers, sequence 1 was mixed immediately after the *Salmonella*-positive batch, and 1 feed sample and 4 surface swabs were still positive for *Salmonella* after sequence 1. This resulted in a low level of contamination of 0.8 cfu/g and 0.1 cfu/cm^2 of *Salmonella* for feed and manufacturing surfaces, respectively. There was also a low level of contamination after manufacturing sequence 1 in the mixers that had manufactured the chemically treated batches. One sample of feed and 1 manufacturing surface of sequence 1 were positive for *Salmonella* after sequence 1 in mixers previously flushed with the commercially available essential oil blend. The feed sample had a *Salmonella* Enteritidis of 0.7 cfu/g, but mean surface contamination rates were lower than the 10 cfu/cm^2 detectable limit. None of the feed samples and 4 of the manufacturing surface swabs were *Salmonella*-positive after sequence 1 in mixers that had previously been flushed with rice hulls treated with 10% MCFA. Although 4 samples were positive, they had a low level of contamination, as the mean *Salmonella* contamination from manufacturing surfaces was 0.1 cfu/cm^2. No feed samples or surface swabs collected after sequence 2 had detectable *Salmonella*.

These results indicate that flushing can reduce *Salmonella* contamination within a mixer, similar to its mechanistic way to reduce drug carryover in medicated feed manufacturing. This is in agreement with data reported by Gebhardt et al. (2016), where sequencing of feed through a mixer and bucket elevator was effective at reducing the porcine epidemic diarrhea virus in swine feed.

The low levels of *Salmonella* residue in feed or on surfaces after sequence 1, but not in the chemically treated batch, may have been impacted by sampling sensitivity because only 12 samples were collected from the 3 mixers. However, we hypothesize that the finding wasbecause of, at least in part, contaminated dust residue. Swabs were collected in targeted locations and not swabbed over the same spot after each sequence. As such, it is plausible that *Salmonella* contamination was denatured by the chemicals during the chemically treated batch but still viable in low levels in the sampling location during sequence 1. Dust collected from animal food contact surfaces has been previously identified to carry pathogenic biological hazards and is therefore one of the highest risks for cross contamination during feed manufacturing (Gebhardt et al., 2016). Owing to the high quantity of airborne particulates in animal food manufacturing facilities, *Salmonella* contamination of such dust may cause it to be a widespread mechanism for hazard transmission. Previously, the impact of contaminated dust has been evaluated in an animal food manufacturing facility. After manufacturing a batch of feed containing *Enterococcus faecium*, nearly all animal food and nonanimal food contact surfaces were positive for the surrogate (Huss et al., 2015). Similar results were observed regarding the role of a viral hazard by Schumacher et al. (2016). Both experiments demonstrated how the quantity of organic material through dust can be specifically challenging for sanitary animal food manufacturing. Huss et al. (2015) also determined that physical cleaning was not effective in reducing the bacteria on environmental surfaces. Highly aggressive procedures were required to completely decontaminate the animal food manufacturing surfaces, including the use of liquid chemical sanitizers and heat.

Previous research has demonstrated that sanitizing animal food contact surfaces with liquids is highly effective but not easily feasible in animal food manufacturing facilities because of their dry bulk systems, the potential for sanitizers to cause corrosion of processing equipment, and the facilities' prevalence for high organic material or dust on manufacturing surfaces (Huss et al. 2015). An evaluation of liquid sanitizers and chemical treatments on stainless steel surfaces has demonstrated that the concentrated form of the proprietary MCFA blend used in this experiment is effective at reducing *Salmonella* Typhimurium (6.6 cfu/cm^2 log reduction; Muckey et al., 2015). The same MCFA blend has been demonstrated to reduce the quantity of postprocessing *Salmonella* serovar Typhimurium contamination if 2% is applied to swine feed before its inoculation with bacteria (Cochrane et al., 2016). One limitation of this product is its proprietary nature and limited availability for manufacturing facilities.

A commercially available alternative with similar properties is the dry essential oil blend used in this experiment. Both products showed promise to reduce the numerical quantity of detectable *Salmonella* in animal food or on surfaces when they were included as flushes; but the 0.8- and 0.1-log reduction in animal food or on surfaces were not significant ($P > 0.05$) compared with those of the control.

For the first time, this study demonstrated how animal food manufacturing surfaces can be contaminated with *Salmonella* Enteritidis after manufacturing a *Salmonella*-positive batch of poultry feed. It is possible for contaminated surfaces to then subsequently adulterate succeeding feed batches. The use of sequencing and using chemically treated flush material may help reduce this potential. Additional research is necessary to further evaluate the role of sequencing and dry sanitizers when *Salmonella* biofilm are formed on manufacturing surfaces.

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

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