Evaluation of surface sanitation to prevent biological hazards in animal food manufacturing

by

Mary Beth Muckey

B.S., Kansas State University, 2015

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Approved by:

Major Professor
Dr. Cassandra Jones

Copyright

Abstract

Animal food manufacturing facilities need to evaluate biological hazards within their facility due to their severity and probability to cause illness or injury in humans or animals. Control of biological hazards, including *Salmonella* and Porcine Epidemic Diarrhea Virus (PEDV), in animal food manufacturing facilities may require a preventative control to mitigate the risk of the hazard. Thermal processing is an effective point-in-time control, but does not prevent cross-contamination during drying, cooling, and packaging/load-out of animal food. Therefore, it may be appropriate to sanitize surfaces to prevent cross-contamination of animal food during manufacturing. The objective of the first experiment was to evaluate surface decontamination strategies for Porcine Epidemic Diarrhea Virus (PEDV) using chemical disinfectants to reduce viral RNA on various manufacturing surfaces. Concentrated liquid formaldehyde and sodium hypochlorite reduced the quantity of viral PEDV RNA on all tested surfaces. Rubber belting from a bucket elevator retained the most PEDV RNA, while the polyethylene tote bag retained the least. In the second experiment, surface decontamination was evaluated for *Salmonella* Typhimurium using liquid and dry chemical sanitizers on various manufacturing surfaces. Surfaces treated with concentrated commercial formaldehyde had no detectable *Salmonella* after treatment, and surfaces treated with medium chain fatty acids (MCFA) had at least a 4-log reduction compared to the control. The dry commercial acidulant, sodium bisulfate, was the most effective dry sanitizer tested, but had limited efficacy depending on surface type.

Experiment 3 further tested the application of two chemical sanitizers against *Salmonella* Enteritidis on residual surface and feed contamination in pilot-scale mixers. Manufacturing sequence, but not treatment impacted feed and surface contamination of *Salmonella* Enteritidis.

Specifically, 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 concentrated commercial essential oil blend or rice hulls treated with 10% MCFA. Low levels of *Salmonella* residues were observed from feed and surfaces manufactured after Sequence 1, but no residues were observed by Sequence 2. This data suggests that sequencing of feed during manufacturing can reduce *Salmonella*-positive contamination within animal food and on manufacturing surfaces, particularly after the second batch or with the use of chemical treatments. In summary, liquid sanitizers have been shown to be effective at reducing *Salmonella* spp. and PEDV contamination on a variety of animal food manufacturing surfaces, but application and practicality may be limited.

Table of Contents

List of Tables

Acknowledgements

I would like to acknowledge my friends and family for supporting me on my many endeavors and to my son, Troy for being my ray of sunshine. I would like to thank everyone who contributed to the work in this thesis. Without my colleagues, professors, family and friends it would not have been possible. Thank you to Drs. Cassandra Jones, Jason Woodworth and Steve Dritz for their support and guidance throughout the research and writing of this thesis. Lastly, I would like to thank my husband, Josh Muckey, and parents, Charles Garzillo and Angela Raulsten, who have always believed in me and supported me throughout my life and college career.

Chapter 1 - The evaluation of surface disinfectants and mitigation strategies to reduce the risk of microbiological hazards in pet food and animal feed manufacturing facilities.

ANIMAL FOOD SAFETY HAZARDS

The introduction of new regulations, such as the Food Safety Modernization Act, and new biological hazards that can be passed through animal food, such as Porcine Epidemic Diarrhea Virus (PEDV), have created interest for biological hazard control and decontamination strategies within animal food manufacturing facilities. Each facility that manufactures, processes, packs, or stores finished animal food or ingredients that will be consumed by animals in the U.S. must identify these potential hazards and evaluate their severity and probability in a food safety plan (21 C.F.R. § 507.31). If the facility determines the hazard's combination of severity and probability requires a preventive control, then that hazard must be significantly minimized or prevented through a preventive control (21 C.F.R. § 507.34). Unfortunately, there is limited information available to help facilities determine the severity or probability of biological hazards within animal food intended for different species. Information is extremely limited to help determine possible control strategies. Therefore, the objective of this review is to serve as a resource for animal food manufacturing facilities to identify and develop control strategies for biological hazards in animal food.

The term 'hazard' is defined by 21 C.F.R. § 507.3 as, "any biological, chemical (including radiological), or physical agent that has the potential to cause illness or injury in humans or animals." Facilities that manufacture, process, pack, or hold animal food must identify and evaluate known or reasonably foreseeable hazards (21 C.F.R. § 507.31). A hazard is

considered to be a ‘known or reasonably foreseeable hazard’ if it is “known to be, or has the potential to be, associated with the facility or the animal food.” According to 21 C.F.R. § 507.33(d), there are 10 items that must be considered during this identification and evaluation process, which include: 1) formulation of the animal food; 2) condition, function, and design of the facility and equipment; 3) raw materials and other ingredients; 4) transportation practices; 5) manufacturing/processing procedures; 6) packaging activities and labeling activities; 7) storage and distribution; 8) intended or reasonably foreseeable use; 9) sanitation, including employee hygiene; and 10) any other relevant factors such as the temporal nature of some hazards (21 C.F.R. § 507.33(d)).

While the facility must determine on its own if a hazard is known or has the potential to be associated with it based on the items listed above, there are resources available to aid with the determination if the hazard is associated with a type of animal food. These resources can include previous recalls, withdrawals, reportable food registry reports, or scientific literature for a specific type of animal food. While chemical and physical hazards are important to consider, the scope of this review is to focus on summarizing the biological hazards associated with different types of animal foods.

A biological hazard addressed by specific U.S. regulation was Bovine Spongiform Encephalopathy (BSE). Prions associated with BSE have been shown to be transmitted through mammalian protein if fed back to ruminant animals. To prevent this occurrence and limit BSE incidence within the US, the FDA published an initial rule in 1997 and strengthened regulation in 2008 (21 C.F.R. § 589.2000 and 21 C.F.R. § 589.2001). This is considered to be one of the most successful rules implemented by FDA, with a high level of compliance and stabilization of the

disease, allowing the return of the US to 'negligible risk' status according to the World Health Organization (*30*).

While BSE was the first potential feed-transmitted biological hazard in the US, recent focus has moved to *Salmonella* spp. The FDA publishes an annual report describing foods that have resulted in Reportable Food Registry (RFR) reports. These are items that any human or animal food, "for which there is a reasonable probability that the use of, or exposure to, such article of food will cause serious adverse health consequences or death to humans or animals" (*13*). Of the 114 RFR reports associated with animal food in the five published annual reports, 49 are due to *Salmonella* spp. contamination in pet food (*13*). This makes it by far the most substantial hazard quantified by FDA in recent years.

Notably, all 49 RFR reports associated with *Salmonella* spp. were due to contamination in pet food. This is predominantly due to the manner in which FDA considers *Salmonella* spp. as an adulterant. For example, the FDA Compliance Policy Guide 690.800: *Salmonella* in Food for Animals describes FDA's current thinking that any finished pet food contaminated with any serotype of *Salmonella* is considered to be adulterated because of its propensity to be a direct human contact food and potentially impact human health (*8*). Alternatively, finished livestock feed is only considered adulterated if it contains a serotype of the bacteria known to cause illness in the animal for which it is intended. Of the more than 2,600 different serotypes of *Salmonella*, only a few are known to cause illness or injury in specific animal species. Those serotypes and their affected animal species are listed in Table 1.1. The occurrence of these specific serotypes in livestock feed is limited. None of those listed have been linked to an RFR in the 6 published annual reports available. Furthermore, only *Salmonella* Enteritidis has been demonstrated to be in the 25 most common serotypes found in animal feed or ingredients according to an FDA

survey (*29*). All other serotypes have a relatively low level of occurrence. Still, presence of any level of the specified serotype, or any serotype at all in pet food, is deemed to be an adulterant in that animal food if it will not undergo further heat processing or a subsequent commercial kill step.

Table 1.1. *Salmonella* as a biological hazard of concern in animal food[abc]	
Poultry	*Salmonella* Pullorum, *Salmonella* Gallinarum, or *Salmonella* Enteritidis
Swine	*Salmonella* Choleraesuis
Sheep	*Salmonella* Abortusovis
Horse	*Salmonella* Abortusequi
Dairy and Beef	*Salmonella* Newport or *Salmonella* Dublin
Pets (including dogs, cats, and aquarium fish)	All serotypes of *Salmonella*
[a.]Table adapted from FDA Compliance Policy Guide Sec. 690.800 *Salmonella* in Food for Animals, 2013. [b.]Additional serotypes will be evaluated on a case-by-case basis. [c.]Animal food will not subsequently undergo a commercial heat step or other commercial process that will kill the *Salmonella.*	

While *Salmonella* spp. is the primary biological hazard of concern in animal food manufacturing facilities, two other biological hazards have surfaced as possible hazards to be evaluated. Prior to 2015, the pet food industry did not recognize *Listeria monocytogenes* as a potential hazard in its products. However, 7 RFR reports were reported from July 2015 to July 2016, all of which occurred in raw, fresh, and frozen pet foods (*13*). Survey data collected from 196 raw dog and cat foods revealed that 8% contained *Salmonella* spp. and 16% tested positive for presence of *Listeria monocytogenes*. Thus, facilities producing finished raw, fresh, or frozen pet foods without a thermal kill step are under increased scrutiny to ensure the safety of their

products through other control measures including Current Good Manufacturing Practices, other processing controls, supply-chain applied controls, and sanitation controls.

While *Salmonella* spp. and *Listeria monocytogenes* have focused on transmission through pet food, another biological hazard has caused harm in swine feed. Recently, feed has been recognized as one of many potential vectors that may transmit Porcine Epidemic Diarrhea Virus (PEDV; *10, 39, 46*). Biosecurity measures have been implemented at many swine feed mills to prevent the entry of PEDV through ingredients, transportation, and people (*4*). Furthermore, dust has been recently shown to cause cross-contamination within feed mills, which may contaminate animal feed manufacturing surfaces (*16*). The transmission of biological hazards has historically been linked to ingredients, but other data supports the role of cross-contamination from animal food contact surfaces, such as the interior of conveyors, bucket elevators, bins, and floors (*10, 16, 22, 24, 25, 36*).

Control of biological hazards, such as *Salmonella* spp., *Listeria monocytogenes*, and PEDV, require a multi-pronged biosecurity plan to 1) minimize hazard entry; 2) control hazards via process controls; and 3) prevent cross-contamination prior to packaging or consumption by an animal. These strategies will be briefly described.

CONTROL OF ANIMAL FOOD SAFETY HAZARDS

Minimization of Entry

Hazards can be significantly minimized or prevented through activities associated with Current Good Manufacturing Practice, the use of designated ingredients and known suppliers, or through more strenuous supply-chain applied controls. The methods of appropriate control vary based on the facility and the type of animal food that it manufactures, processes, packs or holds.

Current Good Manufacturing Practice

Many successful strategies for reducing risk of biological hazards within the facility can begin with successfully complying with current good manufacturing practice requirements (CGMP). According to the FDA, the goal of CGMP requirements is "to prevent animal food from containing filthy, putrid, or decomposed substances, being otherwise unfit for food, or being prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health" (Preamble, II: Legal Authority; *28*). Sections of the requirements include Personnel, Plant and Grounds, Sanitation, Water Supply and Plumbing, Equipment and Utensils, Plant Operations, and Holding and Distribution. Basic requirements of this section that may limit the entry and occurrence of biological hazards in manufacturing facilities include individuals being qualified for their duties, including understanding the importance of employee health and personal hygiene, the presence of handwashing facilities, the minimization of trash and areas of pest harborage, and the elimination of drips and condensate. The use of toxic materials, including cleaning materials and sanitizers, is allowed, but these materials must be safe for their intended use, labeled, used, and stored properly within the manufacturing facility.

Designated Ingredients and Known Suppliers

Another method to reduce the introduction of undesirable microorganisms into a facility is the use of approved suppliers. The acts of using reputable suppliers, monitoring supplier facilities for compliance with expectations, sampling, and testing supplied raw materials can all greatly decrease instances of biological hazards. Some facilities have opted to eliminate problematic suppliers or even entire ingredients that have a historic incidence of a biological hazard of concern. For example, research suggests that some animal proteins can have higher

risks for microbial contamination compared to other ingredient categories (*29*). Surveillance testing conducted by the food and drug administration found that 22.9% of 122 animal food ingredients were positive for *Salmonella* (*15*). Of these, fish meal had the highest incidence of contamination, with 4 of the 5 samples testing positive for *Salmonella* and Escherichia coli, and all samples testing positive for Enterococcus. Similarly, Li (*29*) reported that 66% of animal-derived ingredients tested positive for *Salmonella* spp. from 2002 to 2006, which fell to 41% by 2007 to 2009. Still, this is an extremely high occurrence compared to an 11% incidence rate from the same time periods in plant-derived ingredients. Even more concerning is that many of these ingredients have been associated with resistance to common antimicrobials. Hofacre (*20*) described that of 27 rendered protein ingredients testing positive for *Salmonella* spp., 33% of the *Salmonella* were resistant to tetracycline, 22% to cephalothin, and 11% to ampicillin. Others have pointed to the role of rendered proteins as a potential vector or reservoir of PEDV (*7*).

Due to these incidents, some livestock feed manufacturing facilities have chosen to remove all animal proteins from the feed mill. This practice eliminates a potential vector of biological hazard, but also prevents the use of high quality, relatively inexpensive protein sources. Other facilities, such as pet food manufacturing facilities, cannot remove the ingredient due to its necessary inclusion in the diet. Instead, facilities use known suppliers, purchasing contracts, and letters of assurance to clearly communicate expectations between suppliers and customers. The use of known suppliers and associated activities, such as random sampling or supplier facility audits, can greatly reduce the probability of biological hazard entry through ingredients.

Supply-Chain-Applied Controls

If a facility identifies that a hazard has a severity and probability that requires a preventive control, one way that hazard can be prevented is through a supply-chain-applied control (*23*). According to FDA 21 CFR § 507.105, this is a type of preventive control where the supplier controls the hazard prior to receipt by the receiving facility. This type of preventive control requires a great amount of communication and coordination by both the supplier and customer (21 CFR § 507.110). For example, monitoring of the hazard must occur at the supplying facility, but the receiving facility must have a corrective action plan in place in case product is shipped with the hazard (21 CFR § 507.110). Furthermore, the preventive control requires the receiving facility to identify, conduct, and document verification activities to ensure the supplier is complying with its expectations (21 CFR § 507.115). These verification activities may include an onsite audit, review of the supplier's food safety records, or product testing (21 CFR § 507.130 and 21 CFR. § 507.135). Adding to the complexity is that the supplier is the last entity to grow or manufacture the raw material or ingredient. This means that intermediaries, such as brokers, must disclose the suppliers of their product, and verification activities must be carried out on all suppliers if lots are comingled. Because of the difficulty of communicating, verifying, and documenting these requirements between suppliers and receiving facilities, it is anticipated that many facilities will either use known suppliers to reduce the likelihood of hazard occurrence so they do not require a preventive control, or will control the hazard via process control at their own facility (*23*).

CONTROL HAZARDS VIA PROCESS CONTROLS

Most preventive controls within animal food manufacturing facilities are expected to be process controls, or a control associated with a step that is part of the manufacturing process. These are typically point-in-time mitigation measures, which may include thermal processing, irradiation, or the use of chemical additives to control pH or water activity.

Point-in-Time Mitigants

The pet food industry has relied on thermal processing as a point-in-time mitigant for *Salmonella* control in animal feed ingredients for years. Much of this early research is still relevant today, such as Liu (*31*) who first described D-values of *Salmonella* destruction in an animal food matrix and its relationship with moisture levels. Thermal processing continues to be the most common form of critical control points or process preventive controls for biological hazards, with recent data available for *Salmonella* destruction by preconditioner and PEDV deactivation by pellet mill (*6, 37, 52*). More recently, ingredient manufacturers have looked to the use of ultraviolet light or irradiation to ensure destruction of biological hazards (*11, 50*). Extensive discussions of these are outside the scope of this literature review, but examples have been described by Cochrane (*7*). While highly effective and relatively easy to manage, point-in-time mitigants may be problematic as they do not prevent hazard reoccurrence through recontamination. Thus, animal foods subject to re-contamination with environmental pathogens may need to be treated with chemicals that offer resistance to cross-contamination or all post-mitigation animal food contact surfaces must be cleaned and sanitized, which is not realistic for most of the current animal food industry.

Chemical Mitigants

The use of chemical additives may both significantly minimize or prevent a biological hazard from contaminating animal food, and also provide some protection against subsequent cross-contamination. Formaldehyde-based products, medium chain fatty acids, and essential oils have all demonstrated effectiveness to help prevent post-processing cross-contamination of biological hazards in feeds and ingredients (*3, 5*). Carrique-Mas (*3*) found formaldehyde was the most effective treatment against *Salmonella* in fish meal and meat and bone meal. It also proved to be more effective than formic, propionic, and sorbic acids. There are concerns associated with these mitigants, some of which include that most are not labeled for proactive mitigation, require specialized application equipment, are expensive, and may be harmful to employee health. Added to these issues are that some, such as formaldehyde-based products, may pose customer labeling concerns and require special permitting for use. While effective, many pet food manufacturers cannot justify the use of chemical mitigants in current production. Thus, they must rely on control of biological hazards through traditional thermal processing and prevention of cross-contamination through sanitation controls.

PREVENTION OF CROSS-CONTAMINATION PRIOR TO PACKAGING OR CONSUMPTION BY AN ANIMAL

If a facility identifies a hazard that requires a preventive control and the most appropriate control is a point-in-time mitigant, such as thermal processing, the facility must also take steps to prevent subsequent cross-contamination. This can be accomplished through the use of hygienic zoning and/or surface sanitation.

Hygienic zoning

Hygienic zoning is a method used to control the movement of people, ingredients, and utensils from being a source of cross-contamination. Typically, facilities employ the use of walls, designated maps, and color coding to help employees follow designated traffic flow. A study conducted within an oilseed crushing facility without hygienic zoning reported that the highest concentration of *Salmonella* spp. contamination was from employee shoes; which was greater than the contamination from all other surfaces combined, including brooms, floors, processing surfaces, and rodents (*36*). Even after decontamination of the shoes, contamination existed again within one day (*36*). Crossover areas that had been decontaminated were again contaminated within 1 day, but segregated passage areas remained *Salmonella*-free up to 4 weeks after decontamination. Clearly, employee zoning can be helpful to reduce the cross-contamination of biological hazards as they are passed throughout a facility. However, there are times where surfaces become contaminated and must be sanitized.

Surface Sanitization

Historically, the animal food manufacturing industry has prevented batch-to-batch hazard contamination through planned sequencing or flushing of diets (21 C.F.R. § 255). However, the control measure was designed to reduce contamination of chemical hazards, not biological hazards that have the potential to leave potent residues on equipment or thrive with biofilms. As such, batch-to-batch sequencing alone has shown to be ineffective at eliminating *Enterococcus faecium* (*21*) and Porcine Epidemic Diarrhea Virus (*47*) contamination from equipment. This is likely due to contaminated organic residue and dust (*16*) remaining on surfaces of equipment and conveyors, as well as relatively large quantities of feed remaining in the boot of bucket elevator

conveyors (*47*). In these instances, more strenuous physical cleaning may be necessary. However, the applicability of this option is limited because most equipment in animal food manufacturing facilities was not designed to be clean-in-place. Further complicating biological hazard control on these surfaces is the potential formation of biofilms.

Biofilm formation of pathogenic bacteria may be one method of hazard reintroduction within an animal food manufacturing facility. Biofilms are formed when individual bacterial cells adhere and embed in an extracellular polymeric substance providing a defense mechanism, such as an equipment surface (*33*). Planktonic cells can then respond to form a biofilm. Pathogenic bacteria, including *Salmonella* spp. and *Listeria monocytogenes*, may form biofilms in processing environment (*33*). Their extracellular polymeric matrix is difficult to penetrate for sanitizing. For example, *Salmonella* spp. has been shown to maintain presence on dry surfaces for up to 4 weeks through a biofilm (*18*).

The described challenges in equipment design and biofilm formation must be considered if there is concern that a biological hazard may re-contaminate animal food due to its presence on equipment. In these instances, traditional sequencing/flushing or physical cleaning is not likely to be sufficient for complete control, so a sanitize step is necessary. Sanitize means to adequately treat cleaned surfaces by a process that is effective in destroying vegetative cells of pathogens, and in substantially reducing numbers of other undesirable microorganisms, but without adversely affecting the product or its safety for animals or humans (21 C.F.R. § 507.3). In order to sanitize, surfaces must first be cleaned. Cleaning works by using physical brushing/sweeping or a detergent /cleaning agent/water to physically remove organic debris from surfaces. While physical cleaning in animal food manufacturing facilities has previously been the primary method of hazard mitigation, it is just the first step of mitigating a biological hazard.

Data related to surface sanitization in animal food manufacturing facilities is limited. What is available has typically been reported as a reduction in positive swabs, so the level on contaminated surfaces was not quantified (*51*). Thus, the industry relies on strategies from the human food industry (*34, 35*). Preventative measures utilized by food industry have included coating of surfaces to limit the establishment of vegetative cells or biofilms (*27, 43*). Many of the methods or measures used to sanitize surfaces in the human food industry are impractical for the animal food industry due to cost and liquid application. For example, both Huss (*21*) and Schumacher (*46*) demonstrated that liquid decontamination of animal food manufacturing equipment appears effective, but not practical to implement. This is because most livestock feed manufacturing systems are typically a dry bulk system, so water introduction for cleaning or sanitization may be a source of hazard introduction instead of a reduction mechanism. Generally, a water activity (a_w) level of 0.87 is required for growth of most bacteria pathogens of concern, so introducing a water-based sanitizer may raise the a_w to levels that allow for bacterial growth (*2*). In these instances, dry chemicals may be a more appropriate than liquid surface sanitization.

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 (*32*). Sanitizers can be classified into chlorine and chlorine derivatives, quaternary ammonium compounds, acid-anionic sanitizers, and hydrogen peroxide/ peroxyacetic acids (21 C.F.R. § 178.1010). Other chemicals that may have similar properties and have sanitization properties that may be useful in animal food manufacturing facilities include alcohols, formaldehyde/glutaraldehyde, and medium chain fatty acids.

Chlorine and chlorine derivatives can be used as a broad-spectrum of bactericidal, fungicidal, sporicidal, tuberculocidal, and virucidal control within facilities. The action of the

active ingredient, free chlorine, on microorganisms is not fully understood. Current research indicates a multiplicative of factors that result in hazard destruction, including oxidation of enzymes and amino acids, ring chlorination of amino acids, loss of intracellular contents, decreased uptake of nutrients, inhibition of protein synthesis, decreased oxygen uptake, oxidation of respiratory components, decreased ATP synthesis, nucleic acid replication disruption, and depressed DNA synthesis (*44*). Chlorides include hypochlorite, chlorine gas, and sodium chlorite. Their efficacy decreases with increased pH due to its caustic properties. One consideration is that this high acidity can be hazardous for employers and corrosion to equipment. Notably, hypochlorite solutions may produce the carcinogens bis(chloromethyl) ether when in contact formaldehyde (*14*) and trihalomethane when in contact with hot water (*219*). Hypochlorite and sodium chlorite can be effective detergents and sanitizers, and are known to penetrate biofilms developed by of *Salmonella* (*26, 40*). Because of their potential impact on human health, chloride and its derivatives must be rinsed from surfaces prior to manufacturing food for consumption by animals or humans.

Quaternary ammonium compounds, commonly called ‘quats,’ inactivate energy-producing enzymes, denature essential cell proteins, and disrupt the cell membrane of pathogens (*44*). They are known to be effective sanitizers of fungi, bacteria, and non-enveloped viruses. Their true advantage is an ability to effectively sanitize in the presence of *Salmonella* spp. biofilms and because the presence of organic matter is not as inhibitory to their action as it is to other sanitizers (*1, 40*). For this reason, quats are commonly used in animal rearing facilities. For example, quats have been demonstrated to reduce *Salmonella* spp. by 2 to 3 CFU/cm^2 log on galvanized steel in a poultry barn (*40*). Research also demonstrates their effectiveness to sanitize

stainless steel contaminated with *Listeria monocytogenes* (*38, 42*) and rubber contaminated with *Salmonella* typhimurium (*42*).

Acid-anionic sanitizers, including acetic, benzoic, and propionic acids, have been favored for food processing antimicrobials due to their combination of effectiveness and positive consumer perception. Their mode of action includes acidifying the cytoplasm and disrupting cell membrane organization, which is highly effective for *Salmonella* spp. destruction in human food manufacturing facilities (*3, 41*). Due to their effectiveness and relative safety, organic acid salts have even been used as surface treatment of foods to prevent microbial growth and as a preservative in finished foods (*3, 45, 48*). For example, sodium bisulfate, a dry acidulant, has been demonstrated to prevent *Salmonella* typhimurium cross-contamination when used as a coating on pet food kibble (*22*). While a rinse is not required on surfaces to maintain animal or human food safety, the acids can be corrosive, so surfaces are typically rinsed to maintain equipment integrity.

Hydrogen peroxides/peroxyacetic acids can be used directly on food-contract surfaces without a liquid rinse but have not been shown to be as effective as other chemicals and require higher liquid additions. Their mode of action is that the hydroxyl free radicals disassociate cell membrane, lipids, and DNA of pathogenic microorganisms (*44*). They have been demonstrated to control bacteria, yeasts, fungi, viruses and spores. Hydrogen peroxide products can be stable for long periods of time (less than 2% active ingredient loss per year) at room temperature (*44*).

Alcohols, such as ethanol and isopropanol, are able to destroy undesirable microorganisms through the denaturation of essential proteins and inhibition of nutrients for proliferation. Alcohol antibacterial properties are limited with spore forming bacteria including, *Bacillus* and *Clostridium* (*44*) However, isopropanol has been demonstrated to be effective for

the mitigation of lipid viruses, which include coronaviruses such as feline coronavirus and porcine epidemic diarrhea virus (*44*). Alcohol use has been limited use due to cost and recommended level included high levels (70% ethanol recommended). When evaluating surface disinfectants used by various animal food manufacturing facilities in Norway, alcohol products were more effective (4 log reduction) than acids, aldehydes, peroxides, and chlorine products in suspension tests (*35*). This effectiveness is desirable, as is its limited need for water in order to be effective. However, the chemical must be rinsed from surfaces and completely dried prior to manufacturing animal or human food (*35*).

Formaldehyde and glutaraldehyde are in a class of sanitizers that are available in liquid and gaseous forms and have bactericidal, fungicidal, sporicidal, tuberculocidal, and virucidal control that is commonly used within animal production and health care facilities (*44*). The activity of aldehydes causes alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms and further cell lysis. They are not commonly used within food manufacturing due to consumer perception and labeling concerns, in addition to potential carcinogenic effects to employees if handled improperly. However, they have been demonstrated to be highly effective at preventing *Salmonella* Enteritidis or PEDV cross-contamination when applied directly to animal food (*3, 5, 7*). Aldehydes are also highly effective to decontaminate surfaces in livestock production and handling. Effective formaldehyde gas application of poultry layer houses reduces surface contamination of *Salmonella* spp. (*17*), and PEDV is inactivated by a combination glutaraldehyde/quaternary ammonium chloride disinfectant on aluminum livestock trailers (*49*).

Recent research has utilized antimicrobial properties of medium chain fatty acids for reduction of *Salmonella* typhimurium in animal feeds (*5*). Other fatty acids have been used for inclusion in poultry diets for Salmonellosis. Believed antimicrobial mode of action include

disruption of cell membrane and other essential functions (*12*). Cochrane (*7*) demonstrated efficacy of medium chain fatty acids on reduction of PEDV. Benefits of medium chain fatty acids include high efficacy to formaldehyde products and may provide continued bacteriostatic action due to their amphipathic structure (*12*).

In summary, biological hazards, such as *Salmonella* spp., *Listeria monocytogenes*, and PEDV, are a new category of hazard to a substantial sector of the animal food industry. Their control requires a multi-pronged biosecurity plan to 1) minimize hazard entry; 2) control hazards via process controls; and 3) prevent cross-contamination prior to packaging or consumption by an animal. While research has made strides to address the first two points, there is still limited knowledge as to how prevention of cross-contamination can be employed to the traditional livestock feed industry. Additional research is needed to identify the effectiveness of dry and liquid sanitizers on biological hazards established on animal food manufacturing equipment, particularly when there are differences in types of surfaces. Therefore, the objective of this thesis is to identify the effectiveness of dry and liquid sanitizers on reducing *Salmonella* spp. and PEDV contamination on a variety of animal food manufacturing surfaces

REFERENCES

1. Betty, R. G., J. M. Bieker, and M. D. Tucker. 2005. Agricultural Pathogen Decontamination Technology: Reducing the Threat of Infectious Agent Spread. No. SAND2006-0182. Sandia National Laboratories.
2. Beauchat, L. R., E. Komitopoulou, H. Beckers, R. P. Betts, F. Bourdichon, S. Fanning, H. M. Joosten, and B. H. T. Kuile. 2013. Low-water activity foods: increased concern as vehicles of foodborne pathogens. *J. Food Prot.* 76(1):150-172.
3. Carrique-Mas, J. J., S. Bedford, and R.H. Davies. 2007. Organic acid and formaldehyde treatment of animal feeds to control *Salmonella*. Efficacy and masking during culture. *J. Appl. Microbiol*. 103:88-96.
4. Cochrane, R. A., S. S. Dritz, J. C. Woodworth, C. R. Stark, A. R. Huss, J. P. Cano, R. W. Thompson, A. C. Fahrenholz, and C. K. Jones. 2016. Feed mill biosecurity plans: A systematic approach to prevent biological pathogens in swine feed. *J. Swine Health and Prod.* 24(3):154-164.
5. Cochrane, R. A., A. R. Huss, C. G. Aldrich, C. R. Stark, and C. K. Jones. 2016. Evaluating Chemical Mitigation of *Salmonella* Typhimurium ATCC 14028 in Animal Feed Ingredients. *J. Food Protect*. 79(4):672-676.
6. Cochrane, R. A., L. L. Schumacher, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, Q. Chen, Jianqiang Zhang, P. C. Gauger, R. G. Main, and C. K. Jones. 2015. Effect of Thermal Mitigation on Porcine Epidemic Diarrhea Virus (PEDV)- Contaminated Feed. *Kansas Agricultural Experiment Station Research Reports*. 1(7):2.

7. Cochrane, R. A., M. Saensukjaroenphon, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. F. Bai, Q. Chen, J. Zhang, P. C. Gauger, R. Main, and C. K. Jones. 2016. Evaluating the inclusion level of medium chain fatty acids to reduce the risk of PEDV in feed and spray-dried animal plasma. *J. Anim. Sci*. 94:50-50.
8. Compliance Policy Guide Sec. 690.800 *Salmonella* in Food for Animals; 78 Fed. Reg. 42526.
9. Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventative Controls for Food Animals, 21 CFR 507. 2015.
10. Dee, S., T. Clement, A. Schelkopf, J. Nerem, D. Knudsen, J. Christopher-Hennings, and E. Nelson. 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naïve pigs following consumption via natural feeding behavior: proof of concept. *BMC veterinary research*, *10*(1):1.
11. DeRouchey, J. M., M. D. Tokach, J. L. Nelssen, R. D. Goodband, and S. S. Dritz. Kansas State University Research Foundation, 2003. Use of modified ingredients and feed to improve performance and/or utilization of animals. U.S. patent 6,534,104.
12. Desbois, A. P., and V. J. Smith, 2010. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Applied Microbiology and biotechnology*. 85(6):1629-1642.
13. Food and Drug Administration. 2015. Reportable Food Registry Fourth Annual Report. fda.gov.
14. Gamble, M. R. 1977. Hazard: formaldehyde and hypochlorites. *Laboratory animals*, 11:61..

15. Ge, B., P. C. Lafon, P. J. Carter, S. D. Mcdermott, J. Abbott, A. Glenn, S. L. Ayers, S. L. Friedman, J. C. Paige, D. D. Wagner, and S. Zhao. 2013. Retrospective analysis of Salmonella, Campylobacter, Escherichia coli, and Enterococcus in animal feed ingredients. *Foodborne pathogens and disease*, *10*(8):684-691.
16. Gebhardt J. T., J. C. Woodworth, C. K. Jones, P. C. Gauger, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, M. Muckey, R. A. Cochrane, C. R. Stark, J. Bai J, Q. Chen, J. Zhang, A. Ramirez, R. J. Derscheid, R. G. Main, and S. S. Dritz. 2016. Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on likelihood of porcine epidemic diarrhea virus (PEDV) transmission by swine feed and feed manufacturing equipment. Kansas Agricultural Experiment Station Research Reports. 1:9-10.
17. Gradel, K., J. Jørgensen, J. Andersen, and J. Corry. 2004. Monitoring the efficacy of steam and formaldehyde treatment of naturally Salmonella -infected layer houses. *J. Appl. Microbiol*. 96(3):613-622.
18. Habimana, O., T. Møretrø, S. Langsrud, L. K. Vestby, L. L. Nesse, and E. Heir. 2010. Micro ecosystems from feed industry surfaces: a survival and biofilm study of *Salmonella* versus host resident flora strains. *BMC veterinary research*, 6(1):1.
19. Helms, C., R. Massanari, R. Wenzel, S. A. Streed, M. Pfaller, N. Moyer, N. Hall, W. Johnson, and W. Hausler Jr. 1987. Control of epidemic nosocomial legionellosis: a 5 year progress report on continuous hyperchlorination of a water distribution system. *27th Interscience Conference of Antimicrobial Agents and Chemotherapy*. 349:158.

20. Hofacre, C. L., D. G. White, J. J. Maurer, C. Morales, C. Lobsinger, and C. Hudson. 2001. Characterization of antibiotic-resistant bacteria in rendered animal products. *Avian Diseases, 45*(4):953-961.

21. Huss, A.H., R. A. Cochrane, A. Deliephan, C. R. Stark, and C. K. Jones. 2015. Evaluation of a Biological Pathogen Decontamination Protocol for Animal Feed Mills. *J. Food Prot*. 78:1682.

22. Jeffrey, A. 2016. The role of *Salmonella* in animal food. Manhattan, Kan.: Kansas State University.

23. Jones, C., A. Fahrenholz, B. Miller, and C. Stark, C. 2016. FSPCA Preventative Controls for Animal Food Training Curriculum (1st ed.). *FSPCA*.

24. Jones, F., and K. Richardson. 2004. *Salmonella* in commercially manufactured feeds. *Poult. Res.* 83(3):384-391.

25. Jones, F. T. 2011. A review of practical *Salmonella* control measures in animal feed. *J. Appl. Poult. Res.* 20:102-113.

26. Joseph, B., S. K. Otta, I. Karunasagar, and I. Karunasagar, I. 2001. Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *International journal of food microbiology*. 64(3):367-372.

27. Jullien, C., T. Bénézech, B. Carpentier, V. Lebret, and C. Faille. 2003. Identification of surface characteristics relevant to the hygienic status of stainless steel for the food industry. *Journal of Food Engineering. 56*(1):77-87.

28. Legal Authority: Current Good Manufacturing Practice Regulations. 80 Fed. Reg. 56178.

29. Li, X., L. A. Bethune, Y. Jia, R. A. Lovell, T. A. Proescholdt, S. A. Benz, T. C. Schell, G.

Kaplan, and D. G. McChesney. 2012. Surveillance of *Salmonella* Prevalence in Animal Feeds and Characterization of the *Salmonella* Isolates by Serotyping and Antimicrobial Susceptibility. *Foodborne Pathog. Dis*. 9:692-698.

30. List of BSE risk status: OIE - World Organization for Animal Health. 2016. Retrieved September 12, 2016. Available at: http://www.oie.int/animal-health-in-the-world/official-disease-status/bse/list-of-bse-risk-status/
31. Liu, T. S., G. H. Snoeyenbos, and V. L. Carlson. 1969. Thermal resistance of *Salmonella* senftenberg 775W in dry animal feeds. *Avian Diseases*, 1:611-631.
32. Marriott, N. and R. Gravani. 2006. Principles of food sanitation (5th ed., Food science texts series). New York, N.Y.: Springer
33. McLandsborough, L., A. Rodriguez, D. Pérez-Conesa, and J. Weiss. 2006. Biofilms: At the interface between biophysics and microbiology. *Food Biophysics*. 1(2):94-114.
34. Meyer, B. 2003. Approaches to prevention, removal and killing of biofilms. *International Biodeterioration & Biodegradation*. 51(4):249-253.
35. Møretrø, T., L. K. Vestby, L. L. Nesse, S. E. Storheim, K. Kotlarz, and S. Langsrud. 2009. Evaluation of efficacy of disinfectants against *Salmonella* from the feed industry. *J. Appl. Microbiol*. 106(3):1005-1012.
36. Morita, T., H. Kitazawa, T. Iida, and S. Kamata. 2006. Prevention of *Salmonella* cross-contamination in an oilmeal manufacturing plant. *J. Appl. Microbiol*., 101(2):464-473.
37. Okelo, P. O., D. D. Wagner, L. E. Carr, F. W. Wheaton, L. W. Douglass, and S. W. Joseph. 2006. Optimization of extrusion conditions for elimination of mesophilic bacteria during thermal processing of animal feed mash. *Anim. Feed Sci. and Tech*. 129:116-137.

38. Pan, Y., F. Breidt, and S. Kathariou. 2006. Resistance of Listeria monocytogenes biofilms to sanitizing agents in a simulated food processing environment. Applied and Environmental Microbiology, 72(12): 7711-7717.

39. Pasick, J., Y. Berhane, D. Ojkic, G. Maxie, C. Embury-Hyatt, K. Swekla, K. Handel, J. Fairles, and S. Alexandersen. 2014. Investigation into the role of potentially contaminated feed as a source of the First-Detected outbreaks of porcine epidemic diarrhea in Canada. *Transboundary and Emerging Diseases.* 61(5):397-410.

40. Ramesh, N., S. W. Joseph, L. E. Carr, L. W. Douglass, and F. W. Wheaton. 2002. Evaluation of chemical disinfectants for the elimination of *Salmonella* biofilms from poultry transport containers. *Poultry Science,* 81(6):904-910.

41. Ricke, S. C. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science*, 82(4):632-639.

42. Ronner, A. B. and A. C. Wong. 1993. Biofilm development and sanitizer inactivation of Listeria monocytogenes and Salmonella typhimurium on stainless steel and Buna-n rubber. *J. Food Prot*. 56(9):750-758

43. Rosmaninho, R., O. Santos, T. Nylander, M. Paulsson, M. Beuf, T. Benezech, S. Yiantsios, N. Andritsos, A. Karabelas, G. Rizzo, and H. Müller-Steinhagen, H., 2007. Modified stainless steel surfaces targeted to reduce fouling–evaluation of fouling by milk components. *J Food Engineering*. 80(4):1176-1187.

44. Rutala, W. A., D. J. Weber, and the Healthcare Infection Control Practices Advisory Committee (HICPAC). 2008. Guideline for Disinfection and Sterilization in Healthcare Facilities, Center for Disease Control.

45. Samelis, J., G. K. Bedie, J. N. Sofos, K. E. Belk, J.A. Scanga, and G. C. Smith, G. C. 2005. Combinations of nisin with organic acids or salts to control Listeria monocytogenes on sliced pork bologna stored at 4 C in vacuum packages. *LWT-Food Science and Technology*. 38(1):21-28.
46. Schumacher, L. L., R. A. Cochrane, C. E. Evans, J. R. Kalivoda, J. C. Woodworth, A. R. Huss, C. R. Stark, C. K. Jones, Q. Chen, R. Main, and J. Zhang. 2016. Evaluating the effect of manufacturing porcine epidemic diarrhea virus (PEDV)-contaminated feed on subsequent feed mill environmental surface contamination. *J. of Anim. Sci*.94:77.
47. Schumacher, L.L., R. A. Cochrane, J. C. Woodworth, A. R. Huss, C.R. Stark, C. K. Jones, Q. Chen, R. Main, J. Zhang, P. C. Gauger, and S.S. Dritz. 2016. Utilizing feed sequencing to decrease the risk of porcine epidemic diarrhea virus (PEDV) cross-contamination during feed manufacturing. *J. of Anim. Sci.* 94:76.
48. Smulders, F. J. M. 1995. Preservation by microbial decontamination; the surface treatment of meats by organic acids. *In New methods of food preservation* 1:253-282.
49. Thomas, P. R. 2015. Evaluation of methods for inactivating porcine epidemic diarrhea virus (PEDV) in livestock trailers. Iowa State University.
50. Weaver, E. M., J. M. Campbell, and L. Russell. 1999. U.S. patent 6,004,576.
51. Whyte, P., K. Mc Gill, and J. D. Collins. 2003. A survey of the prevalence of *Salmonella* and other enteric pathogens in a commercial poultry feed mill. *J. Food Saf.* 23(1):13-24.
52. Zhou, T. 2016. Residence Time and Survival Studies for *Enterococcus Faecium* as a Surrogate for *Salmonella* during Preconditioning and Extrusion Processing of Dry Expanded Pet Food. Kansas State University.

Chapter 2 - Using environmental swabbing to quantify the effectiveness of chemical disinfectant to reduce the quantity of PEDV RNA on feed manufacturing surfaces

ABSTRACT

Porcine Epidemic Diarrhea virus (PEDV) is a possible hazard in feed mills that could impact swine health. If the virus enters a feed mill, it quickly becomes widely distributed and is difficult to decontaminate from surfaces. The objective of this study was to evaluate a variety of liquid and dry sanitation treatments that could be used to reduce the amount of PEDV found on feed manufacturing surfaces in feed mills. This experiment was replicated 3 times and was designed as a 5 × 10 factorial with main effects of 5 different feed manufacturing surfaces and 10 sanitizing treatments. Surfaces included stainless steel, plastic, rubber, woven polypropylene tote bag, and sealed concrete coupons (103 cm^2). One mL (1×10^5 $TCID_{50}$/ml) of stock PEDV was applied to each surface and allowed to dry completely for 60 min. Next, chemical treatments were applied for 15 min: 1) no sanitation treatment (control); 2) untreated rice hulls; 3) rice hulls treated with formaldehyde-based commercial product (Sal CURB; Kemin Industries Inc., Des Moines, IA), 4) liquid formaldehyde-based commercial product (Sal CURB; Kemin Industries Inc., Des Moines, IA); 5) dry commercial benzoic acid and eubiotic blend (VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ); 6) liquid ammonium chloride, isopropanol, and hydrogen peroxide-based commercial food-grade sanitizer (DrySan Duo; Ecolab, St. Paul, MN); 7) liquid hydrogen peroxide commercial product (INTERvention; Virox Technologies Inc. Ontario, Canada); 8) liquid quaternary ammonium glutaraldehyde commercial product (Synergize; Preserve International, Reno NV); 9) liquid sodium hypochlorite

commercial sanitizer (Bleach; Clorox, Oakland, CA); and 10) liquid medium chain fatty acid blend of caprylic, capronic, and capric acids. The quantity of PEDV RNA was determined using quantitative reverse transcription PCR (qRT-PCR). All main effects and interaction were highly significant ($P \leq 0.001$). Concentrated liquid Sal CURB was the most effective sanitizer at removing PEDV RNA across surfaces, followed by liquid bleach (42.9, 35.2, and 26.2 CT for Sal CURB, bleach, and untreated control, respectively). Rubber belting obtained from a bucket elevator retained the most PEDV RNA of any tested surface, while the polyethylene tote bag retained the least (28.0 and 31.4 CT for rubber and tote bag, respectively). Additional research is necessary to identify the role of sanitizer on PEDV infectivity, and to develop dry sanitizers capable of removing PEDV mRNA on animal food manufacturing surfaces.

Key Words: PEDV, sanitation, feed manufacturing surfaces

INTRODUCTION

Swine feed mills may be a potential vector for Porcine Epidemic Diarrhea Virus (PEDV) transmission into swine herds (*8, 12, 13*). Recent studies have demonstrated the potential for PEDV to be introduced into the feed mill through ingredients, vehicles, and employees (*2*). Regardless of the method of entry, viral contamination becomes widespread within the manufacturing environment due to cross-contamination from employees, utensils, or even dust (*7, 13*). There are limited options to decontaminate feed mills once viral RNA has become established. Thermal processing, or the use of a pellet mill inactivates the virus at 130°F for 30s (*4*). However, it does not prevent re-contamination from PEDV-contaminated dust or residue on feed manufacturing equipment surfaces after the pelleting process. Chemical sanitizers typically used in human food manufacturing have shown some promise on reducing PEDV RNA on trailer surfaces (*1*). Current industry practices include the use of heat, sodium hypochlorite, or quaternary ammonium/glutaraldehyde combinations to sanitize swine farm surfaces contaminated with PEDV. However, there is limited information regarding their success on reducing viral RNA on feed manufacturing surfaces. Even if there were successful options, there may be limited application of liquid sanitizers due to the inherent dry nature of ingredients and feed. The introduction of water, even in the form of a liquid sanitizer, may actually increase the quantity of other biological hazards if they are not targeted by the sanitizer (*11*). Furthermore, ideal sanitizers would be safe for use in both animal feed and on equipment surfaces. Therefore, the objective of this study was to evaluate the ability of a variety of liquid and dry chemical sanitizers to reduce the quantity of detectable PEDV RNA.

MATERIALS AND METHODS

The experimental treatments were arranged as a 5 × 10 factorial with 5 different feed manufacturing surfaces, 10 chemical treatments, and three replications of each combination.

Surface preparation and viral inoculation.

Surfaces included: 1) stainless steel (stainless steel type 316; Built-So-Well Manhattan, KS); 2) plastic (Dura Bucket National Oats Co. Collinsville, Ill.); 3) rubber (Maxi-Lift Inc. Addison, TX); 4) woven polypropylene tote bag (The MegaSack Corp. Magnolia, AR); and 5) sealed concrete (Quikrete Co. Atlanta, GA). Surface coupons (103.23 cm^2) were representative samples from larger scale manufacturing surfaces. Surface coupons were prepared, inoculated, and treated with chemical as previously described by Bowman (*1*). Briefly, surfaces were sanitized, rinsed, and autoclaved. Next, 1 mL of PEDV (USA/IN/2013/19338; 1×10^5 $TCID_{50}$/ml, initial mRNA CT 20.7) was applied to the surfaces and spread using a cell spreader to cover the entire area. Surfaces were allowed to dry for 60 min. After drying, control samples had a PEDV mRNA concentration of 26.2. This reduction in CT due to inoculation and drying of the virus is similar to that reported by Bowman (*1*) and unpublished data from our laboratory confirming the repeatability of this technique

Surface treatment.

After drying, respective treatment was applied to coupon surface, 1 mL of liquid or 15 g of dry treatment was spread onto each surface for 15 minutes to allow for complete surface coverage. Immediately after dry treatment, excess material was removed by sterile forceps and gently tapping twice. Chemical treatments included: 1) no sanitation treatment (control); 2) untreated rice hulls; 3) rice hulls treated with formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol

blend); 4) liquid formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 5) dry commercial benzoic acid and probiotic blend (VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend); 6) liquid commercial food-grade sanitizer (DrySan Duo; Ecolab, St. Paul, MN; 10.98% isopropyl alcohol, 0.045% hydrogen peroxide, 0.016% alkyl dimethyl benzyl ammonium chloride, 0.007% dodecyl dimethyl ammonium chloride, and 0.005% dioctyl dimethyl ammonium chloride); 7) 3% dilution of liquid hydrogen peroxide commercial product (INTERvention; Virox Technologies Inc. Ontario, Canada; 4.25% hydrogen peroxide); 8) 0.39% dilution of liquid quaternary ammonium glutaraldehyde commercial product (Synergize; Preserve International, Reno NV; 26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde); 9) 10% dilution of liquid sodium hypochlorite commercial sanitizer (Bleach; The Chlorox Company, Oakland, CA; 5 to 10% sodium hypochlorite); and 10) liquid medium chain fatty acid blend of caprylic, capronic, and capric acids as described by Cochrane (*3*; 1:1:1 wt:wt ratio).

Sample collection and statistical analysis.

Surfaces were then swabbed according to Bowman (*1*) to determine residual PEDV contamination using pre-moistened environmental swabs in 5 mL of neutralizing broth (World Bioproducts LLC., Mundelein, IL). Swabs were vortexed and PEDV was quantified using qRT-PCR. Results were analyzed using the SAS version 9.4 (SAS Inst. Ind., Cary, NC). Main effects included surface type and treatment and their interaction. A preplanned contrast included the comparison of dry vs. liquid chemical treatments. Significance was considered at $P \leq 0.05$ and marginally significant from $P > 0.05$ to $P \leq 0.10$.

RESULTS AND DISCUSSION

All main effects and interactions were highly significant ($P \leq 0.001$; Table 2.1 and 2.2). Rubber belting obtained from a bucket elevator retained the most detectable PEDV RNA of any tested surface, while the polyethylene tote bag retained the least (28.0 and 31.4 CT for rubber and tote bag, respectively). Concentrated liquid Sal CURB was the most effective sanitizer at removing detectable PEDV mRNA across surfaces, followed by liquid bleach (42.9, 35.2, and 26.2 CT for Sal CURB, bleach, and untreated control, respectively). The liquid Sal CURB prevented detection of PEDV mRNA (> 45 CT) on plastic, polyethylene tote bag, rubber, and stainless steel. Cement still contained residual PEDV mRNA, even after liquid formaldehyde application, but the sanitizer was still more effective than all other treatments ($P < 0.05$; 36.7 CT). Liquid bleach was most effective at reducing detectable PEDV mRNA on the polyethylene tote bag (43.0 CT), followed by stainless steel, rubber, and plastic ($P < 0.05$; 37.1, 35.6, and 35.0 CT, respectively). However, liquid bleach was least effective on cement ($P < 0.05$; 25.4 CT). All other sanitizers did not influence the detection of PEDV mRNA on any surfaces compared to that detected on the untreated control ($P > 0.05$). Other evaluation of surface disinfectant studies found that oxidizing agent (0.5%) and hypochlorite (bleach; 2.06%) disinfectants were effective in reducing viral detectable mRNA as compared to positive controls, (26.27 and 24.29 vs. 14.46 CT, respectively) Further analysis of oxidizing agent and hypochlorite treatments did not contain viable PEDV during bioassay (*1*). Similar treatment reduction of viral PEDV mRNA of hypochlorite was shown by Bowman (*1*; 24.3 vs. 14.5 CT, for hypochlorite vs. positive control, respectively).

This study evaluated the impact of sanitizers on reducing detectable PEDV mRNA as measured by qRT-PCR and quantified by CT values. This method does not indicate infectivity,

only the presence or absence of viral RNA. Additional research is necessary to identify the role of sanitizer on PEDV infectivity, even if RNA residue remains, and to develop dry non-corrosive sanitizers capable of removing PEDV RNA on animal feed manufacturing surfaces. Added to these issues are that some, such as formaldehyde-based products, may pose customer labeling concerns and require special permitting for use. The transmission of biological hazards has historically been linked to ingredients, but other data supports the role of cross-contamination from animal food contact surfaces, such as the interior of conveyors, bucket elevators, bins, and floors (*8, 13*). Due to wide spread contamination of PEDV, biosecurity measures have been implemented at many swine feed mills to prevent the entry of PEDV through ingredients, transportation, and people (*2*).

Environmental swabbing by Gebhardt (*7*) demonstrates the level of cross contamination of Porcine Epidemic Diarrhea Virus was still present after 4 sequences of uncontaminated feed (44% metal and 100% plastic and rubber surfaces). Dust collected from the animal food manufacturing surface is likely the culprit of this contamination, and has been demonstrated to contain infectious material (*7*). Removing dust alone, however, does not always remove biological hazards, particularly in the case of bacterial hazards. Physical cleaning of animal food manufacturing facilities has shown to be ineffective at reducing concentration of *Enterococcus faecium* from equipment (*9*). This experiment described that highly intensive liquid sanitation and heat was required to completely rid the animal food manufacturing facility from the biological hazard (*9*).

Increased concern of viral transmission through human foods and animal production facilities can provide guidance for sanitation of feed facilities. Escudero (*6*) evaluated two strains of human norovirus and was shown to transfer viral RNA following surface contamination for up

to 42 days. Previous research has evaluated that effective sanitation of trailers has included hydrogen peroxide sanitizer and quaternary ammonium/ glutaraldehyde effective at reducing PEDV infectivity during bioassay (*15*). Currently recommended sanitizers for PEDV include: phenols, peroxygens, hypohloride, and quaternary ammonium/ glutaraldehyde combination. Sanitizing chemicals may vary in type and concentration due to a food contact surface in feed manufacturing and contamination of chemical hazards in animal feed. Thus, there are occasions when animal food manufacturing equipment may require substantial sanitization. Sanitation of surfaces can reduce cross-contamination or can be applied throughout the facility to decontaminate equipment if an undesirable microorganism has been established. Sanitizing with liquid sanitizers typically requires physical cleaning, chemical treatment, rinsing with water, and complete drying.

Control of PEDV in animal food manufacturing should be applied through a multiplicative approach of good manufacturing practices, thermal mitigation, employee zoning, and surface sanitation for prevention of cross contamination (21 C.F.R.§ 507.31). In summary, liquid Sal CURB and liquid bleach were the most effective chemical treatments to reduce the quantity of detectable PEDV RNA, but their application is limited due to their liquid state and potential corrosiveness in animal food manufacturing. Surface type can also influence PEDV mitigation strategies, particularly on rubber belting in bucket elevators or stainless steel, which can be more challenging to decontaminate in animal food facilities. Appropriate surface sanitation should be evaluated by surfaces being sanitized, safety and efficacy of the sanitizer, and capabilities of the animal food manufacturing facility including equipment to be sanitized.

ACKNOWLEDGEMENTS

Appreciation is expressed to the National Pork Board for financial support (awards #15-208).

REFERENCES

1. Bowman, A. S., J. M. Nolting, S. W. Nelson, N. Bliss, J. W. Stull, Q. Wang, & C. Premanandan. 2015. Effects of disinfection on the molecular detection of porcine epidemic diarrhea virus. *Veterinary microbiology*. 179(3):213-218.
2. Cochrane, R. A., S. S. Dritz, J. C. Woodworth, C. R. Stark, A. R. Huss, J. P. Cano, R. W. Thompson, A. C. Fahrenholz, and C. K. Jones. 2016. Feed mill biosecurity plans: A systematic approach to prevent biological pathogens in swine feed. *J. Swine Health and Prod*. 24(3):154-164.
3. Cochrane, R. A., M. Saensukjaroenphon, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. F. Bai, Q. Chen, J. Zhang, P. C. Gauger, R. Main, and C. K. Jones. 2016. Evaluating the inclusion level of medium chain fatty acids to reduce the risk of PEDV in feed and spray-dried animal plasma. *J. Anim. Sci*. 94:50-50.
4. Cochrane, R. A., L. L. Schumacher, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, Q. Chen, Jianqiang Zhang, P. C. Gauger, R. G. Main, and C. K. Jones. 2015. Effect of Thermal Mitigation on Porcine Epidemic Diarrhea Virus (PEDV)- Contaminated Feed. *Kansas Agricultural Experiment Station Research Reports*. 1(7):2
5. Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventative Controls for Food Animals, 21 CFR 507.

6. Escudero, B. I., H. Rawsthorne, C. Gensel, and L. A. Jaykus. 2012. Persistence and transferability of noroviruses on and between common surfaces and foods. *J. Food Prot*. 75:927–935.

7. Gebhardt J. T., J. C. Woodworth, C. K. Jones, P. C. Gauger, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, M. Muckey, R. A. Cochrane, C. R. Stark, J. Bai J, Q. Chen, J. Zhang, A. Ramirez, R. J. Derscheid, R. G. Main, and S. S. Dritz. 2016. Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on likelihood of porcine epidemic diarrhea virus (PEDV) transmission by swine feed and feed manufacturing equipment. Kansas Agricultural Experiment Station Research Reports. 1:9-10.

8. Greiner, L. L. Epidemic diarrhea virus or porcine delta coronavirus ribonucleic acid in areas within feed mills. *Journal of Swine Health and Production*, 24(4).

9. Huss, A.H., R. A. Cochrane, A. Deliephan, C. R. Stark, and C. K. Jones. 2015. Evaluation of a Biological Pathogen Decontamination Protocol for Animal Feed Mills. *J. Food Prot*. 78:1682.

10. Jones, F. T. 2011. A review of practical *Salmonella* control measures in animal feed. *J. Appl. Poult. Res.* 20:102-113.

11. Marriott, N. and R. Gravani. 2006. Principles of food sanitation (5th ed., Food science texts series). New York, N.Y.: Springer.

12. Pasick, J., Y. Berhane, D. Ojkic, G. Maxie, C. Embury-Hyatt, K. Swekla, K. Handel, J. Fairles, and S. Alexandersen. 2014. Investigation into the role of potentially

contaminated feed as a source of the First-Detected outbreaks of porcine epidemic diarrhea in Canada. *Transboundary and Emerging Diseases.* 61(5):397-410.

13. Schumacher, L. L., R. A. Cochrane, C. E. Evans, J. R. Kalivoda, J. C. Woodworth, A. R. Huss, C. R. Stark, C. K. Jones, Q. Chen, R. Main, and J. Zhang. 2016. Evaluating the effect of manufacturing porcine epidemic diarrhea virus (PEDV)-contaminated feed on subsequent feed mill environmental surface contamination. *J. of Anim. Sci*.94:77.
14. Schumacher, L.L., R. A. Cochrane, J. C. Woodworth, A. R. Huss, C.R. Stark, C. K. Jones, Q. Chen, R. Main, J. Zhang, P. C. Gauger, and S.S. Dritz. 2016. Utilizing feed sequencing to decrease the risk of porcine epidemic diarrhea virus (PEDV) cross-contamination during feed manufacturing. *J. of Anim. Sci.* 94:76.
15. Thomas, P. R. 2015. Evaluation of methods for inactivating porcine epidemic diarrhea virus (PEDV) in livestock trailers. Iowa State Universit

TABLES

Table 2.1. Main effects of chemical treatments and feed manufacturing surfaces to reduce the quantity of detectable PEDV RNA with environmental swabbing[1]

	Quantity of PEDV, CT
Surface	
Cement	30.0[ab]
Plastic	28.5[bc]
Polyethylene tote bag	31.4[a]
Rubber	28.0[c]
Stainless steel	28.9[bc]
Chemical treatment	
Untreated control	26.2[c]
Untreated rice hulls	26.7[c]
Commercial formaldehyde-treated rice hulls (2 kg/ton)[2]	26.2[c]
Concentrated commercial formaldehyde[2]	42.9[a]
Concentrated dry commercial benzoic acid and probiotic blend[3]	27.9[c]
Ready-to-use liquid commercial food-grade sanitizer[4]	26.2[c]
3% dilution of liquid hydrogen peroxide commercial product[5]	26.5[c]
0.39% dilution of liquid quaternary ammonium/glutaraldehyde commercial product[6]	28.4[c]
10% dilution of liquid sodium hypochlorite commercial sanitizer[7]	35.2[b]
Concentrated liquid medium chain fatty acid blend[8]	27.4[c]
P=	
Surface	0.001
Treatment	< 0.0001
Surface × treatment	0.001
SEM	
Surface	0.60
Treatment	0.85
Surface × treatment	1.91

[1]This experiment was conducted in a 5 × 10 factorial with 3 replicates per treatment.
[2] Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol blend.
[3]VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend.
[4]DrySan Duo; Ecolab, St. Paul, MN; 10.98% isopropyl alcohol, 0.045% hydrogen peroxide, 0.016% alkyl dimethyl benzyl ammonium chloride, 0.007% dodecyl dimethyl ammonium chloride, and 0.005% dioctyl dimethyl ammonium chloride.
[5]INTERvention; Virox Technologies Inc. Ontario, Canada; 4.25% hydrogen peroxide
[6]Synergize; Preserve International, Reno NV; 26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde.
[7] Bleach; The Chlorox Company, Oakland, CA; 5 to 10% sodium hypochlorite.
[8] Caprylic, capronic and capric acids in 1:1:1 custom blend described by Cochrane et al., 2015, 2016.
[abc]Means with different superscripts differ ($P < 0.05$).

Table 2.2. Interaction of chemical treatments × feed manufacturing equipment surfaces to reduce the quantity of detectable PEDV RNA with environmental swabbing[1]

	Surface type				
	Cement	Plastic	Polyethylene tote bag	Rubber	Stainless steel
Chemical treatment					
Untreated control	27.5^{fghij}	26.7^{fghij}	28.3^{fghij}	23.8^{j}	24.6^{ij}
Untreated rice hulls	31.2^{defg}	24.6^{ij}	28.9^{fghij}	24.3^{j}	24.5^{ij}
Commercial formaldehyde-treated rice hulls (2 kg/ton)[2]	30.3^{defgh}	24.2^{j}	28.5^{fghij}	23.7^{j}	24.5^{ij}
Concentrated commercial formaldehyde[2]	36.7^{bc}	45.0^{a}	43.0^{a}	45.0^{a}	45.0^{a}
Concentrated dry commercial benzoic acid and probiotic blend[3]	30.6^{defgh}	26.1^{ghij}	29.8^{efghi}	26.4^{fghij}	26.3^{ghij}
Ready-to-use liquid commercial food-grade sanitizer[4]	27.9^{fghij}	24.9^{ij}	28.3^{fghij}	24.7^{ij}	26.0^{ghij}
3% dilution of liquid hydrogen peroxide commercial product[5]	27.7^{fghij}	25.4^{hij}	27.8^{fghij}	24.7^{ij}	27.2^{fghij}
0.39% dilution of liquid quaternary ammonium/glutaraldehyde commercial product[6]	31.7^{cdef}	27.1^{fghij}	29.7^{efghi}	26.3^{ghij}	27.3^{fghij}
10% dilution of liquid sodium hypochlorite commercial sanitizer[7]	25.4^{hij}	35.0^{bcde}	43.0^{a}	35.6^{bcd}	37.1^{b}
Concentrated liquid medium chain fatty acid blend[8]	31.1^{defg}	26.3^{ghij}	27.4^{fghij}	26.0^{ghij}	26.0^{ghij}
P=	0.001				
SEM	1.91				

[1]This experiment was conducted in a 5 × 10 factorial with 3 replicates per treatment.
[2] Sal CURB; Kemin Inc., Des Moines, IA; 30% formaldehyde and 10% propionic acid/methanol blend.
[3]VevoVitall and CRINA; DSM Nutritional Products Inc., Parsippany, NJ; 96% benzoic acid and 4% probiotic blend.
[4]DrySan Duo; Ecolab, St. Paul, MN; 10.98% isopropyl alcohol, 0.045% hydrogen peroxide, 0.016% alkyl dimethyl benzyl ammonium chloride, 0.007% dodecyl dimethyl ammonium chloride, and 0.005% dioctyl dimethyl ammonium chloride.
[5]INTERvention; Virox Technologies Inc. Ontario, Canada; 4.25% hydrogen peroxide.
[6]Synergize; Preserve International, Reno NV; 26.0% alkyl dimethyl benzyl ammonium chloride and 7% glutaraldehyde.
[7] Bleach; The Chlorox Company, Oakland, CA; 5 to 10% sodium hypochlorite.
[8] Caprylic, capronic and capric acids in 1:1:1 custom blend described by Cochrane et al., 2015, 2016.
[abcdefghijkl]Means with different superscripts differ ($P < 0.05$).

Chapter 3 - The evaluation of liquid and dry chemical treatments to reduce *Salmonella* contamination on animal food manufacturing surfaces

ABSTRACT

Recent research has confirmed that *Salmonella* can be isolated from animal food, ingredients, and animal food manufacturing surfaces. Currently, there is limited data regarding the sanitation of animal food manufacturing surfaces. Therefore, the objective of this experiment was to evaluate the effects of nine chemical treatments to reduce *Salmonella* Typhimurium contamination on various manufacturing surfaces. This experiment was designed in a 9 × 5 factorial with nine chemical treatments and five surfaces. The nine chemical treatments included: 1) no inoculation or sanitation treatment (negative control), and those inoculated with *Salmonella* typhimurium and treated with 2) no sanitation treatment (positive control), 3) ground corn , 4) liquid commercial formaldehyde, 5) liquid food-grade sanitizer, 6) liquid medium chain fatty acid blend of caprylic, capronic and capric acids (MCFA), 7) dry commercial calcium propionate, 8) dry commercial acidulant, and 9) dry commercial benzoic acid. The five surfaces included 1) stainless steel, 2) plastic, 3) woven polypropylene tote bag, 4) rubber belt, and 5) rubber tire. Plastic had greater *Salmonella* growth in the positive control than the polypropylene tote bag, with other surfaces being intermediate ($P < 0.05$). Surfaces treated with concentrated commercial formaldehyde had no detectable *Salmonella* after treatment, and surfaces treated MCFA had at least a 4-log reduction compared to the control ($P < 0.05$). The dry commercial acidulant was the most effective dry sanitizer tested, but still only resulted a 0.9- to 2.7-log

reduction compared to the control for plastic, polyethylene tote bag, rubber, and stainless steel, respectively, with no impact on *Salmonella* concentration on rubber tires ($P < 0.05$). While most effective in this experiment, liquid sanitizers have limitations in a dry, bulk systems. In summary, liquid 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 which can be more challenging sanitation within animal food facilities.

INTRODUCTION

Salmonellosis globally effects over one million people, with 380 human deaths each year in the United States (*2*). With recent changes in the farm-to-fork initiative by the Food and Drug Administration, animal food manufacturing facilities have placed a greater emphasis on controlling biological hazards, such as *Salmonella*. (21 C.F.R. § 507.3). It has been demonstrated that *Salmonella* and other pathogens may be potentially introduced into facilities through ingredients and employees (*8, 15, 18*). Thermal processing, such as extrusion or pelleting, can eliminate or reduce biological hazards in animal food (*5, 19*). However, post-processing cross-contamination can occur during the manufacturing, storage, and transportation of the finished product through dust, air or employee handling which can cause residual contamination in finished product processing areas (*13*). One way to prevent post-processing cross-contamination is by sanitizing post-processing surfaces. However, there is little data to evaluate the efficacy of various sanitizers on animal food manufacturing surfaces (*10, 18*). What is available is extrapolated from human food manufacturing knowledge, and tends to be focused on liquid

sanitizers. While liquid sanitation has been shown to be effective against biological hazards, including biofilm forming bacteria, it is challenging in a traditional animal food manufacturing facility, which is typically a dry bulk system not designed with clean-in-place equipment (*3, 22*). There are a variety of surfaces within these facilities, and there is no published data evaluating the efficacy of sanitizers for use in animal food manufacturing facilities on varying surface types. Therefore, the objective of this experiment was to identify successful sanitizing treatments 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 -80ºC and inoculated into 10 mL of trypticase soy broth (Difco, Becton, Dickinson, and Company, Franklin Lakes, NJ) for 24 hours at 37°C. Next, samples were streak plated onto tryptic soy agar (Difco, Becton, Dickinson, and Company, Franklin Lakes, NJ) plates held at 35 ± 2°C for 24 ± 2 hours. Single colonies were then used to inoculate trypticase soy broth and were incubated for 35 ± 2°C for 24 ± 2 hours. Next, 1mL of *Salmonella* inoculum broth was pipetted onto sterile coupon surfaces and spread using a cell spreader, as described by Bowman (*1*). Surface coupons included stainless steel representing equipment surfaces (stainless steel 316; Built-So-Well Manhattan, KS), plastic bucket from a bucket elevator conveyor (Dura Bucket National Oats Co. Collinsville, IL), rubber belt from a bucket elevator conveyor (Maxi-Lift Inc. Addison, TX), 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 were 103.23 cm^2 squares, and were placed in sterile petri dishes.

Surface treatment.

Coupons were incubated at 35 ± 2°C for 24 ± 2 hours for biofilm formation of *Salmonella* typhimurium. Next, 1 mL of liquid or 15 g of dry treatment was spread onto each surface for 15 minutes to allow for complete surface coverage. Immediately after dry treatment, excess material was removed by sterile forceps and gently tapping twice. The nine treatments included: 1) no inoculation or sanitation treatment (negative control), inoculated with *Salmonella* with 2) no sanitation treatment (positive control), 3) ground corn , 4) liquid 30% formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA), 5) liquid 0.03% ammonium chloride, 10.89% isopropanol, and 0.045% hydrogen peroxide-based commercial food-grade sanitizer (DrySan Duo; Ecolab, St. Paul, MN), 6) proprietary blend of liquid medium chain fatty acid blend of caprylic, capronic, and capric acids described by Cochrane (MCFA; *4*), 7) dry commercial 97% calcium propionate (SHIELD CA; Kemin Inc., Des Moines, IA), 8) dry commercial acidulant 91.5% sodium bisulfate (Sodium Bisulfate; Jones-Hamilton Co., Walbridge, OH), and 9) dry commercial 99.9% benzoic acid (VevoVitall; DSM Nutritional Products Inc., Parsipanny, NJ). Chemical treatments were also grouped by dry and liquid treatments with dry treatments including SHIELD CA, SBS, and Vevo Vitall, and liquid treatments including Sal CURB, DrySan Duo, and MCFA.

Sample plating and enumeration.

After residue of chemical treatments 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 (*7*) and vortexed prior to dilution (*1, 7*). 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 hours, and then enumerated.

Statistical analysis.

Data was analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Inst. Ind., Cary, NC) as a completely randomized design with the main effects of surface and treatment, the interaction of treatment × surface, and a pre-planned contrast of dry vs. wet chemical treatments. There were 3 replicates per treatment. All results were log transformed and presented as *Salmonella* CFU/cm^2. Difference were considered statistically significant at $P < 0.05$, and marginally significant at $P < 0.10$.

RESULTS AND DISCUSSION

Salmonella mitigation of animal food manufacturing surfaces should include several strategies including minimization of entry, point-in-time mitigation, and post-processing cross-contamination. One method to reduce post-processing contamination is by ensuring post-processing equipment surfaces are not contaminated with biological hazards. Dust collected from the animal food manufacturing surface is likely the culprit of this contamination. Removing dust alone, however, does not always remove biological hazards, particularly in the case of bacterial hazards. Physical cleaning of animal food manufacturing facilities has shown to be ineffective at reducing concentration of *Enterococcus faecium* from equipment (*11*). This experiment described that highly intensive liquid sanitation and heat was required to completely rid the animal food manufacturing facility from the biological hazard (*11*). Thus, there are occasions

when animal food manufacturing equipment may require substantial sanitization. Sanitation of surfaces can reduce cross-contamination or can be applied throughout the facility to decontaminate equipment if an undesirable microorganism has been established. Sanitizing with liquid sanitizers typically requires physical cleaning, chemical treatment, rinsing with water, and complete drying. These activities are typically not practical for animal food manufacturing facilities, so dry sanitizers may be a more practical method if found to be effective.

All main effects and interactions were highly significant ($P \leq 0.001$). Effective sanitizers included commercial formaldehyde, commercial food-grade sanitizer, MCFA, and dry commercial acidulant reduced *Salmonella* concentration compared to the positive control ($P < 0.05$, Table 3.1). The most effective treatment was commercial formaldehyde, where 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* concentration from surfaces compared to the positive control, with MCFA resulting in a 5.8-log mean reduction and the ready-to-used liquid commercial food-grade sanitizer resulting in a 2.9-log reduction compared to the positive control. Previous research evaluating the impact of formaldehyde and MCFA on *Salmonella* within animal feed ingredient has shown to be highly effective to prevent cross-contamination in animal foods treated with the chemical prior to inoculation (*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 vs. 6.7 CFU/cm^2; $P < 0.05$). The only dry treatment that reduced ($P < 0.05$) *Salmonella* concentration below the positive control level was the dry commercial acidulant, which resulted in a 1.3-log reduction. Liquid sanitation were effective including commercial formaldehyde, medium chain fatty acid

blend, and food-grade sanitizer as compared to dry treatments (2.9-6.7 CFU/cm^2 log reduction; $P < 0.0001$).

Surface type also impacted *Salmonella* concentration ($P = 0.001$), with plastic and stainless steel having greater mean *Salmonella* concentration in the positive controls compared to rubber tires, rubber belt, and polypropylene tote bag. (Table 3.1, $P < 0.05$; 4.2, 4.5 versus 4.0, 3.5, 3.3 CFU/cm^2). Previous research has shown that buna-n-rubber and polyethylene coating has shown bacteriostatic and hydrophobic action to *Salmonella*, *Listeria* monocytogenes and protein substrates (*14, 21*).

Effect of chemical treatment on plastic surface.

Plastic surfaces in animal food manufacturing facilities are common in bucket elevator conveyors and utensils, such as shovels. Plastic elevator buckets, which were 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 begin smooth, like in the coupon sampled, but are typically gouged during normal equipment wear, which may provide additional harborage for undesirable microorganisms. Initial *Salmonella* concentration on plastic surfaces were one of the highest surfaces tested, and significantly higher than on polyethylene tote bag (7.4 versus 5.8 log CFU/cm^2; $P < 0.05$; Table 3.2). Other surfaces had intermediate *Salmonella* concentration on the positive control samples. Liquid commercial formaldehyde, food-grade sanitizer, MCFA, and the dry commercial acidulant reduced *Salmonella* concentration 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 desire a 1-log or 90% reduction in *Salmonella* (*17*). These dry acids are typically

less effective than liquids due to the potential formation of biofilms, but have greater consumer appeal and practicality for implementation compared to their liquid counterparts (*12*), Sodium bisulfate is generally recognized as safe and can be used as an animal food ingredient. Thus, its use in animal food manufacturing is readily available and practicle for application. The weak acid salt dissociates to have a 2-phase action: first lowering the pH to limit bacterial growth, and second the desiccation of cytoplasm for a bactericidal effect that is effective in human foods (*12*). The product has been shown to reduce *Salmonella* contamination and reduce cross-contamination when applied as a coating to pet food kibble, and thus has promising results as a sanitizer (*12*). The 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 rubber belt surfaces.

Similar to plastic surfaces, rubber presents specific concern due to rubber being used on bucket elevators. Rubber belts in these conveyors typically becomes cracked and pitted, which can further develop additional surfaces to harbor biological hazards. Previous research has demonstrated that rubber surfaces can resist sanitation by increased growth of certain bacteria including *Listeria monocytogenes* but was less bacteriostatic when compared with *Salmonella typhimurium* (*21*). Treating rubber with commercial formaldehyde and MCFA resulted in the surface having no detectable growth of *Salmonella* in our study ($P < 0.05$; 6.1 CFU/cm^2 log reduction vs. positive control). Commercial food-grade sanitizer was also an effective treatment for reduction of *Salmonella* on rubber compared to the positive control ($P < 0.05$; 3.2 CFU/cm^2 log reduction). All dry treatments had similar *Salmonella* as the control ($P > 0.05$).

Effect of chemical treatment on polypropylene tote bag.

Introduction of biological hazards can occur when animal food manufacturing facilities reuse bags to store or transport animal food or when transportation bags 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 significantly lower than on plastic surfaces ($P < 0.05$; 5.8 versus 7.4 CFU/cm^2, Table 3.2). Notably, these bags contain woven polypropylene plastic, which made the inoculation, sanitizer treatment, and swabbing more challenging. Still, 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 other treatment reduced *Salmonella* concentration on polyethylene tote bags compared 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. While its positive control had one of the highest concentrations of *Salmonella* concentration 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 control ($P > 0.05$). Møretrø (*18*) demonstrated that the most effective chemical at reducing *Salmonella* Senftenberg 1702-1 and *S.* Agona 71-3, dried on stainless steel animal food manufacturing surfaces was 70% ethanol (>4 log reduction) as compared to commercial acids, aldehyde, peroxygens, and chloride products. This sanitizer is highly effective and a common chemical treatment in laboratory settings, but impractical to implement for wide scale animal food manufacturing facilities because residues require rinsing with water and complete drying prior to later manufacturing (*18*).

Effect of chemical treatment on tires.

While not part of the traditional animal food manufacturing environment, vehicle tires are frequently within animal food manufacturing facilities and drive over exposed ingredient pits while unloading animal food. As such, their contamination may lead to cross-contamination of other surfaces or animal food. Some facilities have taken steps to sanitize vehicle tires prior to entering facilities in order to limit their impact as a potential vector. Again, the most effective sanitizing treatment to remove *Salmonella* contamination included commercial formaldehyde and MCFA of *Salmonella* ($P < 0.05$; 6.6 and 6.1 CFU/cm^2 log reduction, respectively). No other sanitizer reduced *Salmonella* contamination from the treatment ($P > 0.05$).

Prior to applying a sanitizer treatment to any surfaces, 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 that will allow 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 during production of porcine epidemic diarrhea virus and *Enterocuccus faeciuim* (*9, 11*). Control of *Salmonella* within a facility should consider microbial growth requirements including water activity, and neutral pH, and wide temperature range (*20*). Altering microbial conditions can reduce *Salmonella* contamination by use of thermal mitigation, reducing water activity, or acidifying usually with a sanitizer.

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 (*17*). Several sanitizers used in this study are highly corrosive, and can cause metal pitting and degradation causing further niche for harborage of bacteria. Corrosiveness of the sanitizers in this experiment were not measured and was outside the scope of 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 include measures for equipment corrosiveness.

In summary, animal food manufacturing surfaces are able to be highly contaminated with *Salmonella* typhimurium, with plastic being more susceptible to polyethylene tote bags. The physical action of unground corn without chemical treatment did not reduce *Salmonella* typhimurium concentration from animal food manufacturing surfaces, Concentrated commercial formaldehyde product highly effective at reducing *Salmonella* contamination to undetectable levels on all tested surfaces. Treatments, 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 of 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.

While this study yielded valuable data as a starting point to identify potentially effective sanitizers, 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

REFERENCES

1. Bowman, A. S., J. M. Nolting, S. W. Nelson, N. Bliss, J. W. Stull, Q. Wang, & C. Premanandan. 2015. Effects of disinfection on the molecular detection of porcine epidemic diarrhea virus. *Veterinary microbiology*. 179(3):213-218.
2. Centers for Disease Control and Prevention. Oct. 1, 2015. *Salmonella*. Accessed Sept. 21 2016. Available at: http://www.cdc.gov/salmonella/indes.html.
3. Cochrane, R. A., S. S. Dritz, J. C. Woodworth, C. R. Stark, A. R. Huss, J. P. Cano, R. W. Thompson, A. C. Fahrenholz, and C. K. Jones. 2016. Feed mill biosecurity plans: A systematic approach to prevent biological pathogens in swine feed. *J. Swine Health and Prod*. 24(3):154-164.
4. Cochrane, R. A., A. R. Huss, C. G. Aldrich, C. R. Stark, and C. K. Jones. 2016. Evaluating Chemical Mitigation of Salmonella Typhimurium ATCC 14028 in Animal Feed Ingredients. *J. Food Protect*. 79(4):672-676.
5. Cochrane, R. A., L. L. Schumacher, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, Q. Chen, Jianqiang Zhang, P. C. Gauger, R. G. Main, and C. K. Jones. 2015. Effect of Thermal Mitigation on Porcine Epidemic Diarrhea Virus (PEDV)- Contaminated Feed. *Kansas Agricultural Experiment Station Research Reports*. 1(7):2.
6. Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventative Controls for Food Animals, 21 CFR 507. 2015.
7. Davidson, C. A., Griffith, C. J., Peters, A. C., & Fielding, L. M. 1999. Evaluation of two methods for monitoring surface cleanliness—ATP bioluminescence and traditional hygiene swabbing. *Luminescence,* 14(1):33-38.

8. Ge, B., P. C. LaFon, P. J. Carter, S. D. McDermott, J. Abbott, A. Glenn, S. L. Ayers, S.L. Friedman, J. C. Paige, D. D. Wagner, S. Zhao, P. F. McDermott and M.A. Rasmussen. 2013. Retrospective Analysis of Salmonella, Campylobacter, Escherichia coli, and Enterococcus in Animal Feed Ingredients. *Foodborne Pathogens and Disease*. 10:684.
9. Gebhardt J. T., J. C. Woodworth, C. K. Jones, P. C. Gauger, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, M. Muckey, R. A. Cochrane, C. R. Stark, J. Bai J, Q. Chen, J. Zhang, A. Ramirez, R. J. Derscheid, R. G. Main, and S. S. Dritz. 2016. Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on likelihood of porcine epidemic diarrhea virus (PEDV) transmission by swine feed and feed manufacturing equipment. Kansas Agricultural Experiment Station Research Reports. 1:9-10.
10. Habimana, O., T. Møretrø, S. Langsrud, L. K. Vestby, L. L. Nesse, and E. Heir. 2010. Micro ecosystems from feed industry surfaces: a survival and biofilm study of *Salmonella* versus host resident flora strains. *BMC veterinary research*, 6(1):1.
11. Huss, A. H., R. A. Cochrane, A. Deliephan, C. R. Stark, and C. K. Jones. 2015. Evaluation of a Biological Pathogen Decontamination Protocol for Animal Feed Mills. *J. Food Prot.* 78:1682.
12. Jeffrey, A. 2016. The role of *Salmonella* in animal food. Manhattan, Kan.: Kansas State University.
13. Jones, F. T. 2011. A review of practical *Salmonella* control measures in animal feed. *J. Appl. Poult. Res.* 20:102-113.

14. Löpez, G. P., B. D. Ratner, C. D. Tidwell, C. L. Haycox, R. J. Rapoza, and T. A. Horbett. 1992. Glow discharge plasma deposition of tetraethylene glycol dimethyl ether for fouling-resistant biomaterial surfaces. *Journal of biomedical materials research*, *26*(4):415-439.
15. Maciorowski, K., F. Jones, S. Pillai, and S. Ricke. 2004. Incidence, sources, and control of food-borne *Salmonella* spp. in poultry feeds. *World's Poultry Science Journal*, 60(4):446-457.
16. Mani-Lopez, E., H. S. García, and A. López-Malo. 2012. Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Research International*. 45(2):713-721.
17. Marriott, N. and R. Gravani. 2006. Principles of food sanitation (5th ed., Food science texts series). New York, N.Y.: Springer
18. Møretrø, T., L. K. Vestby, L. L. Nesse, S. E. Storheim, K. Kotlarz, and S. Langsrud. 2009. Evaluation of efficacy of disinfectants against *Salmonella* from the feed industry. *J. Appl. Microbiol*. 106(3):1005-1012.
19. Okelo, P. O., D. D. Wagner, L. E. Carr, F. W. Wheaton, L. W. Douglass, and S. W. Joseph. 2006. Optimization of extrusion conditions for elimination of mesophilic bacteria during thermal processing of animal feed mash. *Anim. Feed Sci. and Tech*. 129:116-137.
20. Podolak, R., E. Enache, W. Stone, D. G. Black, and P. H. Elliott. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of Salmonella in low moisture foods. *J. Food Prot.* 73:1919-1936.

21. Ronner, A. B., and A. C. Wong. 1993. Biofilm development and sanitizer inactivation of Listeria monocytogenes and Salmonella typhimurium on stainless steel and Buna-n rubber. *J. Food Prot*. 56:750-758.
22. Steenackers, H., K. Hermans, J. Vanderleyden, and S. C. De Keersmaecker. 2012. *Salmonella* biofilms: an overview on occurrence, structure, regulation and eradication. *Food Research International*, 45(2):502-531.

TABLES

Table 3.1 Main effect of chemical treatments and surface type to reduce the *Salmonella* concentration on feed manufacturing equipment surfaces with environmental swabbing[1].

	Quantity of *Salmonella* Log_{10}/cm^2
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,2]
Positive control	6.7[a]
Untreated ground corn	6.1[ab]
Concentrated liquid commercial formaldehyde[3]	NG[e, 2]
Ready-to-use liquid commercial food-grade sanitizer[4]	3.8[c]
Concentrated liquid medium chain fatty acid blend[5]	0.9[d]
Concentrated dry commercial calcium propionate[6]	6.0[ab]
Concentrated dry commercial acidulant[7]	5.4[b]
Concentrated dry commercial benzoic acid[8]	6.2[a]
P=	
Surface	0.001
Treatment	<0.0001
Surface x Treatment	0.001
Dry vs Liquid Treatment	<0.0001
SEM	
Surface	0.19
Treatment	0.26
Surface x Treatment	0.58

[1] This experiment was conducted in a 5 × 9 factorial with 3 replicates per treatment.
[2] NG, no growth of *Salmonella* detected after 24 h of incubation.
[3] Commercial 30% formaldehyde (Sal CURB; Kemin Inc., Des Moines, IA.)
[4] Commercial 0.03% ammonium, 10.89% chloride isopropanol and 0.045% hydrogen peroxide sanitizer (DrySan Duo; ECOLAB, St. Paul, MN)
[5] Medium chain fatty acid blend 1:1:1, Caprilic, capronic and capric acids (Cochrane et al., 2015, 2016)
[6] Commercial 97% calcium propionate (SHIELD CA; Kemin Inc., Des Moines, IA.)
[7] Commercial acidulant (Sodium Bisulfate; Jones-Hamilton Co.)
[8] Commercial 99.9% benzoic acid (VevoVitall; DSM Nutritional Products Inc., Parsipanny, NJ)
[abcde]Means with different superscripts differ ($P < 0.05$)

Table 3.2. Effect of chemical treatment × surface interaction on *Salmonella* inoculated feed manufacturing surfaces [1].

Surface	Plastic	Polyethylene Tote Bag	Rubber	Stainless Steel	Tire
Treatment					
Negative control	NG[2]	NG[2]	NG[2]	NG[2]	NG[2]
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}	7.5^{a}	5.5^{defghi}
Concentrated liquid commercial formaldehyde[3]	NG[2]	NG[2]	NG[2]	NG[2]	NG[2]
Ready-to-use liquid commercial food-grade sanitizer[4]	1.4^{lk}	4.0^{ij}	2.9^{kj}	5.3^{fghi}	5.3^{fghi}
Concentrated liquid medium chain fatty acid blend[5]	1.6^{lk}	1.4^{kl}	NG[2]	0.8	0.5
Concentrated dry commercial calcium propionate[6]	7.0^{abcd}	5.2^{fgh}	5.3^{efghi}	7.0 ^{abcde}	5.7^{cdefgh}
Concentrated dry commercial acidulant[7]	6.4^{bcdefgh}	4.7^{hi}	5.2^{fghi}	4.7^{hi}	6.8^{abcdef}
Concentrated dry commercial benzoic acid[8]	7.5^{a}	5.1^{ghi}	5.1^{ghi}	7.6^{a}	5.5^{defghi}
P=	0.001				
SEM	0.82				

[1] This experiment was conducted in a 5 × 9 factorial with 3 replicates per treatment, presented as log CFU/cm^2.
[2] NG, no growth of *Salmonella* detected after 24 h of incubation.
[3] Commercial 30% formaldehyde (Sal CURB; Kemin Inc., Des Moines, IA.)
[4] Commercial 0.03% ammonium, 10.89% chloride isopropanol and 0.045% hydrogen peroxide sanitizer (DrySan Duo; ECOLAB, St. Paul, MN)
[5] Medium chain fatty acid blend 1:1:1, Caprilic, capronic and capric acids (Cochrane et al., 2015, 2016)
[6] Commercial 97% calcium propionate (SHIELD CA; Kemin Inc., Des Moines, IA.)
[7] Commercial acidulant (Sodium Bisulfate; Jones-Hamilton Co.)
[8] Commercial 99.9% benzoic acid (VevoVitall; DSM Nutritional Products Inc., Parsipanny, NJ)
^{abcdefghijkl}Means with different superscripts differ ($P < 0.05$)

Chapter 4 - Evaluating the roles of surface sanitation and feed sequencing on mitigating *Salmonella* Enteritis contamination on animal food manufacturing equipment

ABSTRACT

The objective of this study was to evaluate the efficacy of flushing surfaces with untreated feed vs. the use of two different 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 status. 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) concentrated commercial product containing a eubiotic blend of essential oils (benzoic acid and blend of essential oils: thymol, eugenol, piperine and other essential oil compounds); or 3) rice hulls treated with a 10% wt/wt addition of a medium chain fatty acid (MCFA; 1:1:1 blend of caprylic, caproic, and capric acids). Each treatment was previously weighed and manufactured prior to inoculation of *Salmonella*. 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. Mixers were not physically cleaned after each sequence, only feed discharged from the mixers. 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 feed (0.7 cfu/g for commercial essential oil blend) and surfaces (0.1 cfu/cm^2 for MCFA) manufactured in Sequence 1, but no residue was observed by Sequence 2. This data suggests 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: *Salmonella*, sanitation, feed manufacturing surfaces

INTRODUCTION

Recent changes in regulation and customer requirements are placing new pressure on the sanitation expectations of animal food manufacturing facilities, particularly those for livestock (*9*). The recently-implemented Food Safety Modernization Act requires animal food manufacturing facilities to evaluate if sanitation controls, such as sanitizing animal food contact surfaces, are necessary to prevent *Salmonella* contamination in the finished product (*6*). 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' biological hazards through the manufacturing system. However, neither method addresses biological hazard residues on surfaces, particularly those that form biofilms resistant to physical cleaning.

With higher emphasis on animal food safety, specifically livestock species, feed mills will now need to reevaluate hazards within their facility to determine if hazard control is necessary (*6, 9*). Most facilities will deem *Salmonella* spp. to not require hazard control due 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 (*10, 14*). 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.

Methods to control biological hazards include Current Good Manufacturing Practices, Process Controls, Supply-Chain-Applied Controls, or Sanitation Controls (*6*). 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 has been generated regarding the efficacy of sanitizers in human food manufacturing facilities, very little data exists 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 two different dry chemical sanitizers on residual surface and feed *Salmonella* Enteritis contamination.

MATERIALS 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 (TSB; Difco, BD) for 24 h at 37°C. Culture was further grown by transferring to fresh TSB to produce a final 1 L 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. The resulting feed was confirmed *Salmonella*-negative, subsampled into 2.2-kg batches, stored, and placed in sealed packages at ambient conditions. *Salmonella*-free rice hulls were mixed with a 10% wt/wt addition of a medium chain fatty acid 1:1:1 blend of caprylic, caproic, and capric acids described by Cochrane (*4*), and subsampled into 2.2-kg batches.

One batch of *Salmonella*-negative feed was mixed in each of the 9 laboratory-scale paddle mixers (Cabela's Heavy Duty Meat Mixer IK-541001; Cabela's Inc., Sidney, NE) for 5 minutes, which was 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 Davidson (*8*) and Bowman (*2*). Briefly, four 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, the *Salmonella* Enteritidis broth inoculum was applied to 50 kg mash poultry diet using a 100-kg paddle mixer (H.C. Davis Sons MFG Co. Inc., Bonner Springs, KS, USA) with a pump sprayer, followed by 5 min of mixing. *Salmonella*-positive feed was discharged from mixer and subsampled into 2.2-kg batches. These batches were then mixed in the 9 laboratory-scale mixers for 5 minutes, samples collected, mixers inverted, and surfaces swabbed using procedures described above. The 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) no treatment (control); 2) concentrated commercial product containing a eubiotic blend of essential oils

(CRINA; DSM Nutritional Products Inc., Parsipanny, NJ); or 3) rice hulls treated with 10% MCFA. Treatment batches were mixed for 5 minutes, samples collected, mixers inverted, and surfaces swabbed using procedures described above. Next, the 9 laboratory-scale mixers were used to manufacture two sequences of *Salmonella*-free feed (Sequence 1 and Sequence 2). Again, feed was mixed for 5 minutes, samples collected, mixers inverted, and surfaces swabbed using procedures described above.

Sample analysis.

After collection of feed and surface swabs, samples were transported on ice to the microbiology lab for serial dilution, plated onto xylose deoxyribose agar, incubated, and enumerated for analysis of *Salmonella* in accordance with FDA Bacteriological Analytical Manual (*1*). The detectable limit for *Salmonella* was 10 colony forming units (CFU per g or swab).

Statistical analysis.

Data were first log transformed and then analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Inst. Ind., Cary, NC) as a completely randomized design with three replicates per treatment. Main effects included treatment (control vs. commercial essential oil blend vs. 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, while contamination on surfaces is presented as CFU/cm^2. Difference were considered statistically significant at $P < 0.05$, and marginally significant at $P < 0.10$.

RESULTS AND DISCUSSION

This study evaluated various methods to reduce the probability that animal food will be a vector for *Salmonella* entry into poultry farms and the human food chain. Manufacturing sequence ($P < 0.0001$), but not treatment ($P > 0.05$) impacted feed or surface contamination of *Salmonella* Enteritidis (Table 4.3). No samples of feed had detectable *Salmonella* after the *Salmonella*-negative batch of feed was manufactured (Table 3.1). 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 below the 10 cfu/cm^2 detectable limit (Table 3.2). Mean *Salmonella* Enteritidis contamination rate across all 9 mixers were 3.63 CFU/g for feed and 1.27 CFU/cm^2 for surface contamination. It is notable that surfaces had more than a 2-log reduction in *Salmonella* Enteritidis contamination compared to the level directly in the feed. However, this study effectively demonstrates that *Salmonella*-positive poultry feed can contaminate animal food manufacturing surfaces and lead to carryover contamination in the next batch. Specifically, 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 by Sequence 2.

Initial surface swabs for *Salmonella* indicated that background *Salmonella* was minimal on mixer surfaces prior to inoculation of *Salmonella*-positive feed (1/ 36 swabs, *Salmonella*-

negative batch). Previous, feed manufacturing surveillance studies for *Salmonella* have identified similar positive swabs within manufacturing (*7*, *17*).

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 (*5*). This is in agreement with data reported by Gebhardt (*11*), where sequencing of feed through mixer and bucket elevator was effective at reducing porcine epidemic diarrhea virus (PEDV) 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. However, we hypothesize that the finding was due, at least in part, to 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 (*11*). Due 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 non-animal food contact surfaces were positive for the surrogate (*13*). Similar results were observed regarding the role of a viral hazard by Schumacher (*19*). Both experiments demonstrated how the quantity of organic material through dust can be specifically challenging for sanitary animal food manufacturing. Huss (*13*) 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.

These results show how an effective surface sanitation control requires both physical cleaning and sanitizing to reduce surface contamination of bacteria (*15*). Prior to sanitizing surfaces, 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 that will allow 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. Inadequate cleaning results in increased water activity and organic material on surfaces, and these nutrients may lead to the proliferation of undesired bacteria (*15*). While thorough cleaning is required, it must be followed by a sanitizer to ensure that loosened vegetative bacteria cells are effectively reduced, otherwise cleaning alone may actually increase cross-contamination. Both steps are necessary as residual organic material from inadequate cleaning can create a buffer with sanitizers, thus reducing sanitizer functionality. These steps produce challenges in the feed industry. Effective cleaning may be difficult because the dry bulk system of feed manufacturing leaves a large quantity of organic material on surfaces. There is limited wet cleaning in these systems, and sanitizing is even less common.

Previous research has demonstrated that sanitizing animal food contact surfaces with liquids is highly effective, but not easily feasible in animal food manufacturing facilities due to 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 (*12, 13,*

16). An evaluation of liquid sanitizers and chemical treatments on stainless steel surfaces has demonstrated that the concentrated form of the MCFA blend used in this experiment is effective at reducing *Salmonella* typhimurium (6.6 CFU/cm^2 log reduction; *16*). The same MCFA blend has been demonstrated to reduce the quantity of post-processing *Salmonella* serovar Typhimurium contamination if 2% is applied to swine feed prior to its inoculation with bacteria (*4*). One limitation of this product is high liquid inclusion and limited availability for manufacturing facilities.

A commercially-available alternative with similar properties is the dry essential oil blend utilized in this experiment. This product is already approved as a livestock feed additive, and has demonstrated abilities to reduce surface biofilms of S*almonella* (*18*). 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 to the control.

While this experiment mimicked commercial animal food manufacturing, its design was unable to evaluate *Salmonella* spp. that had adapted and developed a biofilm on manufacturing surfaces, which is possible in low moisture conditions (*18*). *Salmonella* spp. has been shown to maintain presence on dry surfaces for up to 4 weeks through a biofilm, and contaminate product throughout this entire time (*12*). If this were to occur, it is expected that both physical cleaning and sanitizing would be required to completely mitigate the hazard from manufacturing surfaces (*3*).

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 chemical flushes may help reduce this potential, and sequencing eliminated the risk after the second sequence of feed. Additional research is necessary to further evaluate the role of sequencing and dry sanitizers when *Salmonella* biofilms are formed on manufacturing surfaces.

REFERENCES

1. Andrews, W. H., A. Jacobsen, and T. Hammack. 2015. FDA Bacteriological Analytical Manual, Chapter 5: *Salmonella*. FDA BAM .
2. Bowman, A. S., J. M. Nolting, S. W. Nelson, N. Bliss, J. W. Stull, Q. Wang, & C. Premanandan. 2015. Effects of disinfection on the molecular detection of porcine epidemic diarrhea virus. *Veterinary microbiology*. 179(3):213-218.
3. Chen, Y.H., V. N. Scott, T. A. Freier, J. Kuehm, M. Moorman, J. Meyer, T. Morille-Hinds, L. Post, L. Smoot, S. Hood, and J. Shebuski. 2009. Control of *Salmonella* in low-moisture foods II: Hygiene practices to minimize Salmonella contamination and growth. *Food Prot. Trends*. 29(7):435-445.
4. Cochrane, R. A., A. R. Huss, C. G. Aldrich, C. R. Stark, and C. K. Jones. 2016. Evaluating Chemical Mitigation of *Salmonella* Typhimurium ATCC 14028 in Animal Feed Ingredients. *J. Food Protect*. 79(4):672-676.
5. Current Good Manufacturing Practice for Medicated Feeds. 21 C.F.R.§ 225, 2011.
6. Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventative Controls for Food Animals, 21 C.F.R.§ 507, 2013.
7. Davies, R.H., and A.D. Wales. 2010. Investigations into *Salmonella* contamination in poultry feedmills in the United Kingdom. *J. Appl. Microbiol*. 109:1430-1440.
8. Davidson, C. A., Griffith, C. J., Peters, A. C., & Fielding, L. M. 1999. Evaluation of two methods for monitoring surface cleanliness—ATP bioluminescence and traditional hygiene swabbing. *Luminescence,* 14(1):33-38.
9. FDA Compliance Policy Guide Sec. 690.800 *Salmonella* in Food for Animals, 2013.

10. Ge, B., P. C. LaFon, P. J. Carter, S. D. McDermott, J. Abbott, A. Glenn, S. L. Ayers, S.L. Friedman, J. C. Paige, D. D. Wagner, S. Zhao, P. F. McDermott and M.A. Rasmussen. 2013. Retrospective Analysis of *Salmonella*, *Campylobacter*, *Escherichia* coli, and *Enterococcus* in Animal Feed Ingredients. *Foodborne Pathogens and Disease*. 10: 684.
11. Gebhardt J. T., Woodworth J. C., Jones C. K., Gauger P. C., Tokach, M. D., DeRouchey J. M., Goodband, R. D., Muckey M., Cochrane R. A., Stark C. R., Bai J., Chen Q., Zhang J., Ramirez A., Derscheid R. J., Main R. G., and Dritz S. S. 2016. Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on likelihood of porcine epidemic diarrhea virus (PEDV) transmission by swine feed and feed manufacturing equipment. In Kansas State University Swine Day. Kansas State University.
12. Habimana, O., T. Møretrø, S. Langsrud, L. K. Vestby, L. L. Nesse, and E. Heir. 2010. Micro ecosystems from feed industry surfaces: a survival and biofilm study of *Salmonella* versus host resident flora strains. *BMC veterinary research*, 6(1): 1.
13. Huss, A.H., R. A. Cochrane, A. Deliephan, C. R. Stark, and C. K. Jones. 2015. Evaluation of a Biological Pathogen Decontamination Protocol for Animal Feed Mills. *J. Food Prot*. 78:1682.
14. Li, X., L. A. Bethune, Y. Jia, R. A. Lovell, T. A. Proescholdt, S. A. Benz, T. C. Schell, G. Kaplan, and D. G. McChesney. 2012. Surveillance of *Salmonella* Prevalence in Animal Feeds and Characterization of the *Salmonella* Isolates by Serotyping and Antimicrobial Susceptibility. *Foodborne Pathog. Dis.* 9:692-698.
15. Marriott, N., and R. Gravani,. 2006. Principles of food sanitation, 5th Ed. Springer Science & Business Media. New York, N.Y.

16. Muckey, M. B., A. R. Huss, and C. K. Jones. 2016. The evaluation of liquid disinfectants to reduce *Salmonella* contamination on animal food manufacturing surfaces. *J. Anim. Sci.* 94(E-Suppl. 2):79. (Abstract.)
17. Jeffrey, A.M., C.K. Jones, C.G. Aldrich, A.R. Huss, and C. Knueven. 2015. Identifying sources of *Salmonella* contamination in animal feed and pet food facilities. American Society of Animal Science Joint Annual Meeting. *J. Anim. Sci*. 91(E2)W95.
18. Podolak, R., E. Enache, W. Stone, D. G. Black, and P. H. Elliott. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low moisture foods. *J. Food Prot*. 73:1919-1936.
19. Schumacher L. L., R. A. Cochrane, C.E. Evans, J. R. Kalivoda, J. C. Woodworth, A. R. Huss, C. R. Stark, C. K. Jones, Q. Chen, R. G. Main, J. Zhang, P. C. Gauger, S. S. Dritz, and M. C. Tokach. 2016. Evaluating the Effect of Manufacturing Porcine Epidemic Diarrhea Virus (PEDV)-contaminated Feed on Subsequent Feed Mill Environmental Surface Contamination. *J. of Anim. Sci*. 94: 77.
20. Soni, K.A., A. Oladunjoye, R. Nannapaneni, M. Schilling, J. L. Silva, B. Mikel, and R. Bailey. 2013. Inhibition and inactivation of *Salmonella* Typhimurium biofilms from polystyrene and stainless steel surfaces by essential oils and phenolic constituent carvacrol. *J. of Food Prot*, 76(2):205-212.

TABLES

Table 4.1. Poultry diet composition (as-fed basis)

Item:	Poultry diet
Ingredient, %	
Wheat	63.72
Soybean 46%	29.77
Soy oil	2.82
Limestone	1.19
Dicalcium phosphate	0.98
L-lysine HCl	0.35
Vitamin and mineral premix	0.30
DL methionine	0.29
Sodium bicarbonate	0.29
Salt	0.14
L-threonine	0.11
Choline chloride	0.03
Natugrain TS[1]	0.01
Naturphos E[1]	0.01
Total	100.00

[1]BASF Corp. Florham Park, NJ 07932, USA

Table 4.2. Impact of feed batch sequencing and chemical treatment on number of positive *Salmonella* Enteritis 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 two *Salmonella*-negative feed sequences to evaluate traditional sequencing vs. two different chemical flushes on preventing batch-to-batch feed and manufacturing surface *Salmonella* contamination. Three treatments were tested: 1) no chemical (control), a commercially-available essential oil blend; or 3) rice hulls treated with a 10% concentration of a medium chain fatty acid blend. 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., Parsipanny, NJ).

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

Table 4.3. Impact of feed batch sequencing and chemical treatment on number of positive *Salmonella* Enteritis 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 two *Salmonella*-negative feed sequences to evaluate traditional sequencing vs. two different chemical flushes on preventing batch-to-batch feed and manufacturing surface *Salmonella* contamination. Three treatments were tested: 1) no chemical (control), 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., Parsipanny, NJ).

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