

Title: Evaluating the effects of chemical treatments to reduce the likelihood of PEDV transmission by feed manufacturing equipment. **NPB #15-208**

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Industry Summary:

The overall objective of this research is to evaluate the effectiveness of various chemical mitigation products applied to different feed manufacturing surfaces on the reduction of porcine epidemic diarrhea virus (PEDV) genetic material to mitigate the risk of disease transmission originating from feed manufacturing facilities. The first experiment was to characterize sanitizer's effectiveness for the surface of materials typically encountered in a feed mill environment. The second experiment was focused on evaluating protocols for reducing PEDV infectivity in the feed manufacturing system where feed contact surfaces are not easily decontaminated using wet cleaning methods. A secondary objective was to evaluate infectivity of dust samples collected during feed manufacturing.

For the first experiment, 5 different feed manufacturing surfaces and 10 sanitizing treatments were evaluated. Surfaces included stainless steel, plastic, rubber, woven polypropylene tote bag, and sealed concrete, which were evaluated by applying PED virus, followed by application of the various sanitizing agents. Quantification of detectable genetic material collected via surface swab was then performed using real time, reverse transcriptase polymerase chain reaction (qRT-PCR). Liquid Sal CURB and liquid bleach were the most effective chemical treatments to reduce the quantity of detectable PEDV RNA, but the practicality of their application in field settings is limited due to their liquid state and potential corrosiveness.

For Experiment 2, medium chain fatty acids (MCFA) and formaldehyde were used to treat rice hulls to disinfect laboratory-scale mixers and a production scale mixer and bucket elevator system. Overall, the use of rice hull flushes effectively reduced the quantity of detectable RNA present after mixing a batch of PEDV-positive feed. Chemical treatment of rice hulls with formaldehyde and 10% MCFA provided additional reduction in detectable RNA. Finally, dust collected after manufacturing PEDV-inoculated feed contains a very high quantity of viral RNA and was found infective, demonstrating it has the potential to serve as a vector for PEDV transmission.

Producer bottom line;

Differences in surface structure and porosity affect the ability of chemical disinfection agents to inactivate and remove genetic material

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Liquid disinfectants including formaldehyde and sodium hypochlorite (bleach) appear to be efficacious PEDV surface disinfection agents; however, practicality of use and safety in feed manufacturing facilities need to be considered.

Using abrasive agents (i.e. rice hulls) reduces cross contamination and treatment of the rice hulls with formaldehyde or MCFA can further reduce risk for manufacturing feed where wet cleaning methods cannot be easily applied.

Dust can contain a high quantity of PEDV genetic material that can cause infectivity and therefore be a vector for PEDV transfer.

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Scientific Abstract:

Two experiments were conducted to determine the effectiveness of chemical mitigation compounds when applied to various feed production surfaces on elimination of porcine epidemic diarrhea virus (PEDV) genetic material. Additionally, treated and untreated rice hull flushes were used to determine if PEDV cross contamination could be prevented utilizing equipment like that used in commercial feed production.

In Exp. 1 different materials used in feed manufacturing surfaces were evaluated using liquid and dry chemicals that could be used in feed mills as sanitizers. Treatments were arranged in a 5×10 factorial arrangement with the main effects of 5 different feed manufacturing surfaces and 10 sanitizing treatments. Surface samples (4×4 in.) included stainless steel, plastic, rubber, woven polypropylene tote bag, and sealed concrete. One mL (1×10^5 TCID₅₀/ml) of stock PEDV was applied to each surface and allowed to dry completely for 60 min. Next, 1 of 10 potential sanitizer treatments was applied: 1) no sanitation (control); 2) untreated rice hulls; 3) rice hulls treated with formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA), 4) liquid formaldehyde-based commercial product (Sal CURB; Kemin Inc., Des Moines, IA); 5) dry commercial benzoic acid and probiotic blend (VevoVital 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) a 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. After treatment, the surfaces rested for 15 min., were then swabbed and the quantity of PEDV RNA was determined using qRT-PCR. There were 3 replicates per treatment. All main effects, interactions, and comparisons were highly significant ($P \leq 0.001$). 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 in field settings due to their liquid state and potential corrosiveness.

In Exp. 2, PEDV infectivity during feed manufacturing was evaluated using untreated rice hulls and rice hulls treated with MCFA or formaldehyde. Initially, feed without evidence of PEDV RNA contamination was inoculated with PEDV. Based on PCR analysis, this feed had a Ct = 30.2 and was confirmed infective in bioassay. After manufacture of PEDV positive feed, untreated rice hulls,

or rice hulls treated with Sal CURB, 2%, or 10% medium chain fatty acid blend (MCFA; 1:1:1 ratio of caproic, caprylic, and capric acid) were flushed through laboratory-scale paddle mixers. For the untreated rice hulls, 3 of 6 samples had detectable PEDV RNA (avg. Ct = 41.4) while 1 of 6 Sal CURB treated rice hull flush samples and 2 of 6 of the 2% MCFA rice hull flush samples had detectable PEDV RNA. However, PEDV RNA was not detected in any of the 10% MCFA rice hull flush samples. Additionally, rice hulls treated with 10% MCFA were mixed and discharged through a production-scale mixer and bucket elevator following manufacturing of PEDV positive feed. In the production scale system, no rice hull flush or feed samples from the mixer following chemically-treated rice hull flush had detectable PEDV RNA. However, one 10% MCFA rice hull sample collected from the bucket elevator discharge spout had detectable PEDV RNA.

Dust collected following mixing of PEDV-contaminated feed had a large quantity of PEDV RNA (avg. Ct = 29.4). Dust collected immediately after the 10% MCFA rice hull flush batch had a reduced quantity of PEDV RNA (Ct = 33.7), and the subsequent feed following the 10% rice hull flush had no detectable PEDV RNA. Pigs inoculated with dust collected after manufacturing PEDV-positive feed were shedding PEDV RNA by 2 dpi and continued to have detectable RNA until necropsy. Dust collected from the 10% MCFA rice hull flush batch or the subsequent batch was not infective.

Overall, it is possible to decontaminate feed manufacturing equipment, but sanitizers that are effective, practical to use, and safe for workers to handle are unavailable. The use of rice hull flushes effectively reduced the quantity of detectable RNA present after mixing a batch of PEDV-positive feed. Chemical treatment of rice hulls with Sal CURB and 10% MCFA provided additional reduction in detectable RNA present in the rice hull flush samples and was not infective via bioassay. Finally, dust collected after manufacturing PEDV-inoculated feed contains a very high quantity of viral RNA and was found infective, demonstrating it has the potential to serve as a vector for PEDV transmission.

Introduction:

A previous National Pork Board-funded project (Woodworth et al., #14-273) discovered that if a batch of feed that is contaminated with PEDV is manufactured using equipment like that utilized in commercial mills, cross-contamination to subsequent batches of feed will occur. Another significant finding from this project was the discovery that wide-spread environmental PEDV contamination will occur, not only in the feed manufacturing equipment, but also the general environment and surfaces found throughout the feed mill. Consequently, there is a need to identify ways to decontaminate the feed mill equipment and environment as well as identify a means to prevent cross contamination from one batch of feed to the next.

One potential method to reduce the risk of cross contamination is to treat feed or ingredients with chemicals, such as medium chain fatty acids (MCFA) or commercial formaldehyde products. Both chemical products have been demonstrated to reduce the likelihood and time of viral contamination when applied directly in the feed matrix (Dee et al., 2014 and Jones et al., NPB #14-158, 2015). Preliminary data from our laboratory suggest that commercial formaldehyde or the previously-tested MCFA blend are effective at complete mitigation of *Salmonella* Typhimurium-contaminated feed manufacturing surfaces, including stainless steel, plastic, rubber, cement and polyethylene tote bags. However, these and other potential chemical products have not been tested on feed mill surfaces to determine if they can prevent PEDV cross contamination risk to the feed mill environment. If successful, these products could provide the first identified means of decontaminating a feed mill, which would be of value for PEDV-related concerns as well as any other pathogen that might use feed as a vehicle of transmission.

Because the feed manufacturing equipment is primarily a closed system, it is close to impossible to properly clean or disinfect the feed-contact surfaces without dismantling all the equipment.

Because this is unrealistic for a commercial mill to consider, we hypothesized that creating a chemical-treated flush that could be passed through the feed equipment would provide a means for internal disinfection of the feed manufacturing system to reduce the risk of cross contamination. We believe that the abrasiveness of rice hulls would lend them to being the ideal carrier for the chemical treatment flush because they would “scratch” the feed contact surfaces and provide better disinfecting potential than other options.

Objectives:

The objectives of this proposal were:

Objective 1: Evaluate the ability of different chemicals to reduce the quantity of PEDV RNA on feed manufacturing equipment surfaces.

Objective 2: Evaluate the ability of a chemical-treated flush to reduce the likelihood of PEDV cross contamination during feed manufacturing.

Materials & Methods:

Two separate experiments were conducted to answer our objectives.

Exp. 1

The experimental treatments were arranged as a 5×10 factorial with 5 different feed manufacturing surfaces and 10 chemical treatments. Each combination was replicated 3 times. 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). 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 (VevoVital 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 (1:1:1 custom blend).

A 4×4 in. coupon of each surface was sanitized, rinsed, and autoclaved. Next, 1 mL of PEDV (USA/IN/2013/19338; 1×10^5 TCID₅₀/ml) was applied to the surfaces and spread using cell spreader to cover the entire area. Surfaces were allowed to dry for 60 min. After drying of PEDV, respective treatment was applied to coupon surface for 15 min.

Surfaces were then swabbed 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 GLIMMIX

procedure of SAS (SAS Institute, Inc., Cary, NC). 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 $P \leq 0.10$.

Exp. 2

General

The experiment was conducted at the Kansas State University Cargill Feed Safety Research Center (FSRC). Prior to the experiment, the FSRC was decontaminated following a standard protocol approved by the Kansas State University Institutional Biosafety Committee. Prior to initiation of the experiment, the FSRC was physically cleaned using compressed air and sweeping, chemically cleaned using a two-step process using a 1:256 dilution of ammonium glutaraldehyde blend (Synergize; Preserve International, Reno, NV) and a 1:32 dilution of sodium hypochlorite solution. The facility was then heated to 140°F for 24 h, then cooled to room temperature at which point the environmental surfaces were sampled (World Bioproducts, Mundelein, IL) and verified devoid of PEDV viral RNA to ensure efficacy of the disinfection procedures prior to initiation of the experiment. After disinfection, the facility was held in containment mode with negative air pressure and High-Efficiency Particulate Arrestance (HEPA) filters designed to prevent contaminated air from leaving the facility. Containment was maintained throughout the experiment and through the post-decontamination procedures using the same procedures described above.

The swine diet (Table 3) used in this experiment was manufactured at the Kansas State University O.H. Kruse Feedmill and was verified to be devoid of PEDV RNA and porcine delta-coronavirus (PDCoV) RNA as determined via qRT-PCR prior to initiation of the experiment. Rice hulls were also analyzed using qRT-PCR prior to the experiment to confirm lack of PEDV genetic material. The production-scale mixer used was a 0.14-yd³ electric paddle mixer (H.C. Davis Sons Manufacturing, model # SS-L1; Bonner Springs, KS) with a mix time of 5 min. Feed was discharged at a rate of approximately 10 lb/min into a bucket elevator (Universal Industries, Cedar Falls, IA) fitted with 74 buckets (114 cm³ each), and then discharged through a 10-inch diameter discharge spout, and collected in plastic biohazard bags. Laboratory-scale stainless steel paddle mixers ($n = 13$; Cabela's Inc., Sidney, NE) were validated for mixer efficiency for 5.5 and 11.0 lb batches using a mix time of 5 min. Validation of mixers prior to the experiment to achieve a coefficient of variation of less than 10% was done following previously described procedures.

Chemical treatment of flush batches and negative samples

Prior to initiation of the experiment, six 5.5 lb chemically-treated rice hull batches were prepared using 2% MCFA blend ($n = 2$; 1:1:1 ratio of caproic, caprylic, and capric acid), 10% MCFA blend ($n = 2$; same ratio of acids used as in 2% blend), or commercial formaldehyde ($n = 2$; Sal CURB, Kemin Industries, Inc.; application rate = 6.5 lb/T). Untreated rice hulls (5.5 lb; $n = 2$) were also weighed and prepared prior to initiation of the experiment. Rice hulls (untreated and chemically-treated) were stored in double lined bags for 48 hours at room temperature (70°F) until initiation of experiment.

Prior to inoculation with PEDV, batches of feed were placed in, mixed, and discharged through both a laboratory-scale mixer and production-scale systems. For the laboratory-scale mixers, 500 g of PEDV negative feed was added to each mixer, rotated for approximately 15 s, then disconnected from the drive unit and inverted in a one-step motion to dispose of feed into a waste container. A small quantity of residual feed remained in each mixer after this systematic priming discharge procedure. Following the priming of each mixer, a 5.5 lb batch of PEDV-negative feed was added to each laboratory-scale mixer and mixed as described above. The mixer then was shut off, drive coupler removed from the drive unit motor, and a subsample was collected from six locations within each mixer for a total sample size of approximately 0.5 lb. The mixer was then fully disconnected and inverted to dispose of feed into a waste container.

After priming and collection of the negative feed sample from the laboratory-scale mixer, the production-scale system was primed and negative sample collected. An 11 lb batch of PEDV-negative feed was added to the production-scale mixer, allowed to mix for approximately 15 seconds, and subsequently discharged into the bucket elevator and was collected at the discharge spout to prime the mixer and fill the boot of the bucket elevator. A 110 lb batch of PEDV-negative feed was then added to the production-scale mixer, mixed for 5 min, and then discharged into the bucket elevator and collected in bags at the discharge spout. A sample of feed was collected from multiple subsample points within the discharged batch of feed.

Laboratory-scale mixer inoculation, flush, and subsequent feed

The viral inoculum was cell culture derived PEDV (USA/IN/2013/19338, passage 9) and had an initial concentration of 4×10^6 TCID₅₀/mL. A 1:10 dilution was performed using phosphate buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY) to create 2,500 mL of 10^5 TCID₅₀/mL viral inoculum. Inoculation of feed to be used in each of the laboratory-scale mixers was performed in 11.0 lb batches using an additional laboratory-scale mixer in which 9.9 lb of PEDV negative feed was added to the mixer and 500 mL of 10^5 TCID₅₀/mL diluted viral inoculum was added to create 11.0 lb of 10^4 TCID₅₀/g inoculated feed. This batch was mixed for 5 min, at which point it was split into two samples using a riffle splitter and weighed into 5.5 lb batches, bagged, and stored in a freezer (10°F) until inoculated into appropriate laboratory scale mixer. This process was repeated three additional times, to create a total of eight 5.5 lb batches of inoculated feed.

After preparation of laboratory-scale mixer inoculated feed, each of 8 laboratory-scale mixers was inoculated with feed, flush step performed, and a subsequent batch of feed was mixed and sampled. For each inoculation, a bagged sample of PEDV-inoculated feed was randomly selected from the freezer and placed into the randomly selected laboratory-scale mixer. Feed was mixed for 5 min, at which point a sample of PEDV-inoculated feed was collected from 6 locations within the mixer. PEDV-inoculated feed was then discarded into biohazard waste bags using a complete inversion of the mixer following the systematic procedure as described above with no tapping or additional cleaning action. The appropriate flush batch was added to the mixer and mixed for 5 min. A sample of the rice hull flush was collected from 6 locations within the mixer as previously described. The remaining flush was then discarded, and a subsequent 5.5 lb batch of PEDV-negative feed was added to the mixer and mixed. After mixing, a sample of the subsequent feed was collected, and remaining feed was discarded. This process was repeated 7 additional times in a random order blocked by repetition number, for a total of 8 laboratory-scale mixers with two replicates of each of the four chemical treatments (untreated rice hulls, Sal CURB-treated rice hulls, 2% MCFA-treated rice hulls, and 10% MCFA-treated rice hulls).

Production scale system inoculation, flush, and subsequent feed

For inoculation of the production scale system, a 9.9 lb batch of PEDV-negative feed was added to a clean laboratory-scale paddle mixer and 500 mL of 10^6 TCID₅₀/mL inoculum was slowly added to create an 11 lb batch of PEDV inoculated feed (10^5 TCID₅₀/g). Upon conclusion of the addition of the virus, the batch was mixed for 5 min to ensure an even mix of virus into the feed inoculum. The PEDV feed inoculum was then added to 99 lb of PEDV-free swine diet in the production scale mixer to create the 110-lb batch of PEDV positive feed (10^4 TCID₅₀/g). The entire batch of PEDV-positive feed was then mixed for 5 min, discharged into the bucket elevator, and collected at the bucket elevator discharge spout in biohazard waste bags. A sample of PEDV-positive feed was collected from multiple locations within the discharged batch of PEDV-positive feed. This sample of PEDV inoculated feed was combined at a 1:1 ratio with PEDV-inoculated feed (also 10^4 TCID₅₀/g) from laboratory scale mixer to create a single PEDV-positive sample.

After inoculation of the production scale mixer, 79.2 lb of ground rice hulls were added directly to the mixer, along with 8.8 lb of MCFA (1:1:1 ratio of caproic, caprylic, and capric acid) to create a

10% MCFA rice hull flush with a similar mixer fill volume as a 110 lb batch of feed. After a 5 min mix time, 6 samples were collected from various locations within the mixer. The rice hull flush batch was then discharged into the bucket elevator and collected at the bucket elevator discharge spout. Samples of discharged flush material were collected at multiple times during discharge to create a single composite sample.

A 110 lb batch of PEDV-negative feed was then added to the production-scale mixer and allowed to mix for 5 min. A 0.5 lb sample was collected from the mixer and remaining feed was discharged into the bucket elevator and collected at the bucket elevator discharge spout. Another 0.5 lb sample was collected from six locations of the bucket elevator to create a single composite sample. Samples were placed on ice and transported to the laboratory for qRT-PCR analysis preparation.

Dust samples were also collected throughout the experiment, including dust collected after mixing of 10^4 TCID₅₀/g inoculated feed in both the laboratory and production scale systems, after mixing of 10% MCFA treated rice hulls in the production-scale mixer, and collected after mixing the subsequent feed following the 10% MCFA rice hull flush. All dust collection surfaces were above the fill level of the mixer; therefore, all collected dust had become airborne before depositing on the collection surfaces. Dust was collected from the same surface after each batch of feed (positive inoculated feed, 10% MCFA rice hull flush, and subsequent PEDV-free feed); therefore, dust collected was produced during the associated mixing process and not from previous manufacturing processes.

Viral RNA Quantification

After sample collection, temporary storage on ice, and transport to the Kansas State University Molecular Diagnostic Research and Development Laboratory, three 50.0 g subsamples of feed from each collection point was added to individual 500 mL high density polyurethane (HDPE) bottles. Rice hull samples from each collection point were subsampled into three 25.0 g samples and added to individual 250 mL HDPE bottles. After subsampling of all feed and rice hull flush samples into appropriate bottles, varying quantities of PBS (100 or 200 mL for rice hull or feed, respectively) were added to each bottle to create a 20% suspension. Bottles were shaken for approximately 10 sec, at which point they were allowed to settle overnight at 39.2°F. On the next day, supernatant was collected and multiple aliquots prepared for further analysis. A total of 4 aliquots from each sample bottle were collected and stored at -4°F until qRT-PCR analysis was performed within 7 d of inoculation on one aliquot per sample bottle. The remaining three samples per bottle were stored at -112°F until further use. Dust samples were subsampled into 1 mL aliquots, and 4 mL of PBS was added resulting in a 20% suspension by volume. Samples were stored in a similar manner to feed and rice hull flush bottles, and supernatant pulled the following day to be analyzed via qRT-PCR. The remaining dust was stored in dry form at -112°F until initiation of the bioassay portion of the experiment.

Polymerase chain reaction (PCR) assays were performed at the Kansas State University Molecular Diagnostic Research and Development Laboratory. Briefly, fifty microliters (μ L) of supernatant from each sample was loaded into a deep well plate and extracted using a Kingfisher 96 magnetic particle processor (Fisher Scientific, Pittsburg, PA) and the MagMAX-96 Viral RNA Isolation kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions with one modification, reducing the final elution volume to 60 μ L. One negative extraction control consisting of all reagents except the sample was included in each extraction. The extracted RNA was frozen at -4°F until assayed by qRT-PCR. Reported values represent threshold cycle time (Ct) at which virus was detected. A greater Ct value indicates more cycles must proceed until viral genetic material was detected, thus representing lower quantities of genetic material in the original sample.

Infectivity Characteristics

Bioassay samples were selected after qRT-PCR analysis, and included a composite positive and negative control sample that had been collected from both laboratory and production-scale mixers, untreated rice hull flush, 2% MCFA rice hull flush, Sal CURB rice hull flush, subsequent feed after the untreated rice hull flush, subsequent feed after Sal CURB rice hull flush, subsequent feed after the 2% MCFA rice hull flush, subsequent feed after the 10% MCFA rice hull flush, 10% MCFA rice hull flush collected from the discharge spout of the production-scale system, and subsequent feed after the 10% MCFA rice hull collected from the discharge spout of the production-scale system. Additionally, dust samples included in the bioassay were those collected from mixing surfaces after manufacture of 10^4 TCID₅₀/g inoculated swine feed, dust collected after the 10% MCFA rice hull flush, and subsequent feed after the 10% MCFA rice hull flush. Supernatant samples were thawed prior to bioassay inoculation at room temperature, beginning approximately 3 h prior to inoculation. Dust samples were prepared by combining the three positive control dust samples into a single, homogeneous positive control dust sample. A total of three, homogeneous, dust samples (positive, 10% MCFA rice hull flush, and subsequent feed dust) were then each split into three 5.2 g aliquots, and then adding 20.8 g PBS to create a 1:5 suspension of dust to total mass, with a volume of approximately 25 mL each. A 1 mL sample of the suspension was sampled for qRT-PCR analysis, and the remaining solution was inoculated into the appropriate pig ($n = 3$ pigs per dust type).

The experimental protocol for the pig bioassay portion of the experiment was reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee. Forty-two crossbred, 10 d-old pigs of mixed sex were sourced from a single commercial, crossbred farrow-to-wean herd with no known prior exposure to PEDV. Pigs received a dose of cefitiofur (Excede, Zoetis, Florham Park, NJ) just prior to transport to the research facility. Upon arrival, piglets were ear tagged, weighed and randomly assigned to bioassay treatment rooms. Fecal swabs were obtained and confirmed negative for PEDV, porcine delta coronavirus (PDCoV) and transmissible gastroenteritis virus (TGEV) using qRT-PCR analysis. To further confirm PEDV negative status, serum was collected and confirmed negative for PEDV antibody by an indirect fluorescent antibody (IFA) assay and TGEV antibody by ELISA conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before inoculation.

Pigs were first segregated by weight and then randomly divided into 14 treatment groups with 3 pigs per group and with similar average weight per group. Pigs from each treatment were housed in separate rooms with independent ventilation systems. Rooms had solid flooring that was minimally rinsed to reduce risk of PEDV aerosols. Pigs were fed liquid milk replacer once daily and offered a commercial pelleted swine diet ad libitum with free access to water. Each of 33 pigs (11 rooms) receiving supernatant samples were administered 20 mL of the PBS supernatant by orogastric gavage using a 10-gauge French catheter 0 days post-bioassay inoculation (dpi). Each of 9 pigs (3 rooms) were inoculated with dust suspensions by administering the separated supernatant via orogastric gavage (approximately 5 to 10 mL), with the remaining solid fraction of the inoculum directly placed in the mouth of each pig at which point they were stimulated to swallow. Rectal swabs were collected daily throughout the bioassay experiment from all piglets, and tested for PEDV RNA via qRT-PCR on d -2, 0, 2, 4, 6, and 7 dpi. Fresh small intestine, cecum, and colon were collected at necropsy at 7 dpi, along with an aliquot of cecal content. One section of formalin-fixed proximal, middle, and distal jejunum and ileum were collected for histopathology. Cecal content was evaluated for presence of PEDV genetic material via qRT-PCR. Tissue was processed and fixed in neutral buffered formalin, embedded, sectioned, and stained with hematoxylin and eosin stain. One section of proximal, middle, and distal jejunum; and three serial sections from the piece of ileum (for a total of six sections of intestine) were evaluated by a veterinary pathologist blind to the treatments.

Statistical Analysis

Data were analyzed using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) to determine differences between the treatments. Pairwise comparisons were used to determine differences among flush strategies, with the model protected by the overall *F*-test. Bottle was included in the model as a random effect. A cycle time value of 45 was used in the statistical analysis for samples not containing detectable genetic material. Results for response criteria were considered significant at $P \leq 0.05$.

Results and Discussion:

Exp. 1

All main effects and interactions were highly significant ($P \leq 0.001$; Table 1 and 2). Rubber belting obtained from a bucket elevator retained the most PEDV RNA of any tested surface, while the least amount of PEDV RNA was detected on the polyethylene tote bag ($P < 0.05$; 28.0 vs. 31.4 CT for rubber vs. tote bag, respectively). Concentrated liquid Sal CURB was the most effective sanitizer at removing PEDV RNA across surfaces, followed by liquid bleach ($P < 0.05$; 42.9 vs. 35.2 CT for Sal CURB vs. bleach, respectively). The liquid Sal CURB prevented detection of PEDV RNA (> 45 CT) on plastic, polyethylene tote bag, rubber, and stainless steel. Cement still contained residual PEDV RNA, even after liquid formaldehyde application, but the sanitizer was still more effective than other treatments ($P < 0.05$; 36.7 CT). Liquid bleach was most effective at reducing PEDV RNA on the polyethylene tote bag (43.0 CT), followed by stainless steel, rubber, and plastic (37.1, 35.6, and 35.0 CT, respectively). Liquid bleach was least effective on cement ($P < 0.05$; 25.4 CT). All other sanitizers did not influence the detection of PEDV RNA on any surface compared to that detected on the untreated control ($P > 0.05$). Due to the performance of liquid Sal CURB and liquid bleach, liquid sanitizers were substantially more effective at reducing the quantity of detectable PEDV RNA compared to dry sanitizers ($P < 0.05$).

Exp. 2

After qRT-PCR analysis, the composite negative feed sample did not have detectable RNA, and composite positive control feed sample contained detectable PEDV genetic material (Table 4). Following a PEDV positive batch of feed in laboratory scale mixers, 50% of the untreated rice hull flush samples had detectable PEDV RNA. The untreated rice hull flush reduced ($P < 0.05$) the quantity of detectable RNA compared to the PEDV positive batch of feed. One Sal CURB treated rice hull flush sample was positive for PEDV genetic material, and 33% of the 2% MCFA rice hull samples had detectable PEDV RNA. Additionally, none of the 10% MCFA rice hull flush samples had detectable PEDV RNA. Chemically-treated rice hull flushes using Sal CURB and 10% MCFA reduced ($P < 0.05$) the quantity of detectable RNA present in the rice hull flush samples compared to the untreated rice hull flush. However, the 2% MCFA rice hull flush did not reduce ($P = 0.215$) the quantity of genetic material compared to the untreated rice hull flush. Importantly, no feed samples after an untreated or chemically-treated rice hull flush had detectable PEDV RNA.

After manufacturing a PEDV-positive batch of feed in the production-scale mixer and bucket elevator, one 10% MCFA rice hull sample collected from the bucket elevator discharge spout had detectable RNA. However, none of the rice hull flush samples collected from the mixer or subsequent feed samples from the mixer or bucket elevator discharge spout had detectable PEDV RNA. The presence of detectable viral RNA in the 10% MCFA-treated rice hull flush sample collected from the bucket elevator discharge spout provides evidence that bucket elevators are a significant source of cross contamination within feed manufacturing systems.

All pigs were free of PEDV genetic material in fecal swabs and PEDV-specific antibodies prior to initiation of the bioassay experiment. On 2 dpi, fecal shedding of PEDV RNA was detected in

positive control pigs. No other flush feed bioassay pigs had detectable RNA in fecal swabs throughout the study or cecal content collected at necropsy.

Dust collected after mixing the positive feed had a large quantity of viral RNA (Table 5). Following the inoculated batch of feed, dust collected immediately following the 10% MCFA rice hull flush batch had a reduced quantity of viral RNA, and subsequent feed following the 10% MCFA rice hull flush did not have detectable RNA. Pigs inoculated with the positive dust collected following mixing of inoculated feed were shedding PEDV by d 2 after oral inoculation and continued to shed through necropsy at 7 dpi. However, pigs inoculated with the dust from the 10% MCFA rice hull flush batches or the subsequent feed batch had no indications of PEDV infection.

Overall for Exp. 2, the rice hull flush effectively reduced the amount of detectable RNA present compared to feed inoculated with PEDV, as expected. Chemical treatment of rice hulls with Sal CURB and 10% MCFA provided additional reduction in detectable RNA present in the rice hull flush samples. Feed manufactured following rice hull flushes did not contain PEDV RNA, therefore utilizing rice hull flushes would be a cost-effective strategy to mitigate the risk of PEDV transmission via the feed manufacturing process. Thus, from a practical standpoint we believe a commercial feed mill would utilize chemically treated rice hulls in the same equipment and flow as if they were manufacturing a batch of feed. This “chemical flush” would disinfect the feed manufacturing equipment, ultimately minimizing cross contamination risk. We envision that the feed mills could utilize this quality control step immediately prior to manufacturing feed for high risk customers (i.e. feed for sow farms) and/or would incorporate this concept in the normal QA/QC and cleaning protocols used at the mill.

Finally, dust collected following the manufacture of PEDV contaminated feed contained a large quantity of PEDV RNA and was infective. Therefore, a high level of caution should be taken to limit and control dust created during feed manufacturing as it may serve as a vector in PEDV transmission.

In conclusion, liquid Sal CURB and liquid bleach were the most effective chemical treatments to reduce the quantity of detectable PEDV RNA on feed manufacturing surfaces, but their application is limited due to their liquid state and potential corrosiveness. Additional research is necessary to identify the role of sanitizer on PEDV infectivity, even if RNA residue remains, and to develop dry sanitizers capable of removing PEDV RNA on swine feed manufacturing surfaces that are not corrosive. The use of rice hull flushes effectively reduced the quantity of detectable RNA present after mixing a batch of PEDV-positive feed. Chemical treatment of rice hulls with Sal CURB and 10% MCFA provided additional reduction in detectable RNA present in the rice hull flush samples and we failed to find evidence of infectivity after flushes were applied. Also, we confirmed dust collected after manufacturing PEDV-inoculated feed contains a very high quantity of viral RNA and was found infective, demonstrating it has the potential to serve as a vector for PEDV transmission.

Table 1. Main effects of different chemical treatments to reduce the quantity of PEDV RNA on feed manufacturing equipment surfaces with environmental swabbing, Exp. 1¹

	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
<i>P</i> =	
Surface	0.001
Treatment	< 0.0001
Surface × treatment	0.001
Dry vs. liquid treatment	< 0.0001
SEM	
Surface	0.60
Treatment	0.85
Surface × treatment	1.91

¹This 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 VevoVital 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).

^{abc} Means with different superscripts differ (*P* < 0.05).

Table 2. Interaction of chemical treatments and feed manufacturing equipment surfaces to reduce the quantity of PEDV RNA with environmental swabbing, Exp. 1¹

	Surface type				
	Cement	Plastic	Polyethylene tote bag	Rubber	Stainless steel
Chemical treatment					
Untreated control	27.5 ^{fg hij}	26.7 ^{fg hij}	28.3 ^{fg hij}	23.8 ^j	24.6 ^{ij}
Untreated rice hulls	31.2 ^{defg}	24.6 ^{ij}	28.9 ^{fg hij}	24.3 ^j	24.5 ^{ij}
Commercial formaldehyde-treated rice hulls (2 kg/ton) ²	30.3 ^{defgh}	24.2 ^j	28.5 ^{fg hij}	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 ^{fg hij}	26.3 ^{ghij}
Ready-to-use liquid commercial food-grade sanitizer ⁴	27.9 ^{fg hij}	24.9 ^{ij}	28.3 ^{fg hij}	24.7 ^{ij}	26.0 ^{ghij}
3% dilution of liquid hydrogen peroxide commercial product ⁵	27.7 ^{fg hij}	25.4 ^{hij}	27.8 ^{fg hij}	24.7 ^{ij}	27.2 ^{fg hij}
0.39% dilution of liquid quaternary ammonium/glutaraldehyde commercial product ⁶	31.7 ^{cdef}	27.1 ^{fg hij}	29.7 ^{efghi}	26.3 ^{ghij}	27.3 ^{fg hij}
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 ^{fg hij}	26.0 ^{ghij}	26.0 ^{ghij}
<i>P</i> =	0.001				
SEM	1.91				

¹This 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 VevoVital 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.

abcde fghijkl Means with different superscripts differ (*P* < 0.05).

Table 3. Diet composition (as-fed basis), Exp. 2

Item:	Swine gestation diet
Ingredient, %	
Corn	79.40
Soybean meal	15.60
Monocalcium phosphate	1.40
Calcium carbonate	1.15
Choice white grease	1.00
Salt	0.50
L-Thr	0.03
Trace mineral premix	0.15
Sow add pack	0.50
Vitamin premix	0.25
Phytase ¹	0.02
Total	100
Calculated analysis, %	
Crude protein	14.0
Crude fiber	2.2
Ether extract	4.0
Ca	0.85
P	0.62
Available P	0.46

¹ HiPhos 2700, DSM Nutritional Products, Parsippany, NJ.

Table 4. Effect of chemically treated rice hull flushes on PEDV RNA detection and infectivity of samples collected in feed manufacturing equipment, Exp. 2¹

Item	Rice hull treatment			
	Untreated	Sal CURB ²	2% MCFA ³	10% MCFA
Prevalence, % positive by PCR				
Negative feed	0.0 (0/3)			
Positive feed	100.0 (3/3)			
Laboratory scale mixer				
Rice hull flush	50.0 (3/6)	16.7 (1/6)	33.3 (2/6)	0.0 (0/6)
Subsequent feed	0.0 (0/6)	0.0 (0/6)	0.0 (0/6)	0.0 (0/6)
Production scale mixer				
Rice hull flush	---	---	---	0.0 (0/3)
Subsequent feed	---	---	---	0.0 (0/3)
Production scale bucket elevator				
Rice hull flush	---	---	---	33.3 (1/3)
Subsequent feed	---	---	---	0.0 (0/3)
Cycle threshold, Ct and bioassay				
Negative feed	45.0 ^a (-) ⁴			
Positive feed	30.2 ^d (+)			
Laboratory scale mixer				
Rice hull flush	41.4 ^c (-)	43.9 ^{a,b} (-)	42.4 ^{b,c} (-)	45.0 ^a
Subsequent feed	45.0 ^a (-)	45.0 ^a (-)	45.0 ^a (-)	45.0 ^a (-)
Production scale mixer				
Rice hull flush	---	---	---	45.0 ^a
Subsequent feed	---	---	---	45.0 ^a
Production scale bucket elevator				
Rice hull flush	---	---	---	42.0 ^{b,c} (-)
Subsequent feed	---	---	---	45.0 ^a (-)

¹ Swine feed was inoculated with porcine epidemic diarrhea virus (PEDV) at a concentration of 10⁴ TCID₅₀/g and passed through laboratory scale paddle mixers, followed by a rice hull flush, and subsequent batch of PEDV negative swine diet. Batch size was 5.5 lb with a mix time of 5 min. (---) means that those treatments were not applied in the production scale mixer and bucket elevator.

² Sal CURB (Kemin Industries, Inc., Des Moines, IA) was added at recommended level of 6.5 lb/T.

³ Medium chain fatty acid blend (1:1:1 ratio of caproic, caprylic, and capric acid) added on a wt:wt basis to ground rice hulls.

⁴ (+) indicates 3/3 pigs were shedding PEDV genetic material at 2 dpi and continued to shed through 7 dpi and cecal content collected at necropsy contained PEDV genetic material while (-) indicates 0/3 pigs had detectable PEDV genetic material in fecal swabs or cecal content and remained negative throughout the full 7 d bioassay.

^{a,b,c} Cycle threshold means lacking common superscript differ ($P < 0.05$). Pooled SEM=0.85.

Table 5. Effect PEDV RNA detection and infectivity in environmental dust samples, Exp. 2¹

Item	Cycle threshold, Ct ²	Infectivity ³
Positive feed dust	29.4	+
10% MCFA rice hull dust	33.7	-
Subsequent feed dust	45.0	-

¹ Dust samples were collected from the laboratory and production mixers from non-feed contact surfaces.

² Positive feed dust, average of $n = 3$, 10% MCFA rice hull dust, $n = 1$; subsequent feed dust, $n = 1$.

³ Evaluated in a 10 d old pig bioassay with 3 pigs per dust type. (+) indicates 3/3 pigs were shedding PEDV genetic material at 2 dpi and continued to shed through 7 dpi and cecal content collected at necropsy contained PEDV genetic material while (-) indicates 0/3 pigs had detectable PEDV genetic material in fecal swabs or cecal content and remained negative throughout the full 7 d bioassay.