Evaluation of the effects of flushing feed manufacturing equipment with chemically treated rice hulls on porcine epidemic diarrhea virus cross-contamination during feed manufacturing[1]

Jordan T. Gebhardt,[†] Roger A. Cochrane,[†] Jason C. Woodworth,[†] Cassandra K. Jones,[†] Megan C. Niederwerder,[‡] Mary B. Muckey,[‖] Charles R. Stark,[‖] Mike D. Tokach,[†] Joel M. DeRouchey,[†] Robert D. Goodband,[†] Jianfa Bai,[‡] Philip C. Gauger,[§] Qi Chen,[§] Jianqiang Zhang,[§] Rodger G. Main,[§] and Steve S. Dritz[‡,2]

[†]Department of Animal Sciences and Industry, College of Agriculture, Manhattan, KS 66506; [‡]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506-0201; [‖]Department of Grain Sciences and Industry, College of Agriculture, Kansas State University, Manhattan, KS 66506; and [§]Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, 50011

ABSTRACT: Various strategies have been proposed to mitigate potential risk of porcine epidemic diarrhea virus (**PEDV**) transmission via feed and feed ingredients. Wet disinfection has been found to be the most effective decontamination of feed mill surfaces; however, this is not practical on a commercial feed production scale. Another potential mitigation strategy would be using chemically treated rice hulls flushed through the feed manufacturing equipment. Therefore, the objective of this study was to determine the effects of medium-chain fatty acids (**MCFA**) or formaldehyde-treated rice hull flush batches as potential chemical mitigation strategies for PEDV during feed manufacturing. Feed without evidence of PEDV RNA contamination was inoculated with PEDV. Based on polymerase chain reaction analysis, this feed had a cycle threshold (Ct) = 30.2 and was confirmed infective in bioassay. After manufacturing the PEDV-positive feed, untreated rice hulls, formaldehyde-treated rice hulls, 2% MCFA- (a 1:1:1 blend of hexanoic, octanoic, and decanoic acid) treated rice hulls, or 10% MCFA-treated rice hulls were flushed through laboratory scale mixers. For the untreated rice hulls, 3 of 6 samples had detectable PEDV RNA, whereas 1 of 6 formaldehyde-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. Then, rice hulls treated with 10% MCFA were mixed and discharged through a production scale mixer and bucket elevator following PEDV-positive feed. No rice hull flush or feed samples from the mixer following chemically treated rice hull flush had detectible PEDV RNA. However, one 10% MCFA rice hull sample collected from the bucket elevator discharge spout had detectible PEDV RNA. Dust collected following mixing of PEDV contaminated feed had detectable PEDV RNA (Ct = 29.4) and was infectious. However, dust collected immediately after the 10% MCFA rice hull flush batch had a reduced quantity of PEDV RNA (Ct = 33.7) and did not cause infection. Overall, the use of rice hull flushes effectively reduced the quantity of detectible RNA present after mixing a batch of PEDV-positive feed. Chemical treatment of rice hulls with formaldehyde or 10% MCFA provided additional reduction in detectible RNA. Finally, dust collected after manufacturing PEDV-inoculated feed has the potential to serve as a vector for PEDV transmission.

Key words: chemical treatment, flush, medium-chain fatty acid, porcine epidemic diarrhea virus, swine

[1]Contribution no. 18-506-J from the Kansas Agric. Exp. Stn., Manhattan, KS 55606-0210. Funding, wholly or in part, was provided by The National Pork Board as project no. 15-208.

[2]Corresponding author: Dritz@ksu.edu

Received May 30, 2018.
Accepted July 20, 2018.

J. Anim. Sci. 2018.96:4149–4158
doi: 10.1093/jas/sky295

INTRODUCTION

Feed manufacturing equipment has been shown to be a potential source of porcine epidemic diarrhea virus (**PEDV**) cross-contamination (Schumacher et al., 2017). Wet disinfection has been found to be the most effective feed mill equipment surface decontamination method (Muckey, 2016). However, this is not practical in most current commercial feed production settings. Methods to chemically or thermally mitigate the risk of PEDV transmission in feed and feed ingredients have been investigated (Cochrane et al., 2015; 2016; 2017). These methods are not universally applicable to all feed manufacturing facilities due to equipment cost or safety concerns. Other research has assessed sequencing batches of PEDV-negative feed following an inoculated batch of feed to assess the effectiveness of reducing the risk of viral transmission (Schumacher et al., 2016a). Although this may be a practical mitigation technique for feed mills to implement, there remains a significant quantity of viral particles on feed-contact surfaces including dust production and distribution throughout the facility (Schumacher et al., 2017). This dust may pose a risk for contamination of subsequent diets. One potential solution is to use chemical mitigants such as formaldehyde or medium-chain fatty acids (**MCFA**) as a periodic flush step within the feed manufacturing process. Rice hulls were selected as the carrier for this chemical flush because of the relatively low cost and high degree of abrasiveness, which may help facilitate the removal of viral contamination on equipment surfaces. Therefore, the objective of this experiment was to determine effects of MCFA- or formaldehyde-treated rice hull flush batches as potential PEDV chemical mitigation strategies during feed manufacturing.

MATERIALS AND METHODS

General

The experiment was conducted at the Kansas State University Feed Safety Research Center (FSRC) in Manhattan, KS. 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 sweeping and compressed air, and then chemically cleaned using a 2-step process of a 1:256 dilution of ammonium glutaraldehyde blend (Synergize; Preserve International, Reno, NV) and a 1:32 dilution of sodium hypochlorite solution using procedures outlined by Huss et al. (2017). The facility was then heated to 60 °C for a minimum of 24 h and cooled to room temperature at which point the environmental surfaces were sampled using swabs (World Bioproducts, Mundelein, IL) moistened with phosphate-buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY) and verified devoid of PEDV viral RNA to ensure efficacy of the disinfection procedures prior to initiation of the experiment. After chemical disinfection, the facility was held in containment mode with negative air pressure and high-efficiency particulate air (**HEPA**) filters preventing contaminated air from leaving the facility. Containment was maintained throughout the experiment and through the post-decontamination procedures.

The swine diet used in this experiment was manufactured at O.H. Kruse Feedmill located at Kansas State University and was verified to be devoid of PEDV and porcine delta-coronavirus (PDCoV) genetic material as determined via quantitative real time polymerase chain reaction (qRT-PCR) prior to initiation of the experiment (Table 1). Rice hulls were also verified to be devoid of detectable PEDV and PDCoV genetic material. The production scale mixer used was a 0.113-m^3 electric paddle mixer (H.C. Davis Sons Manufacturing, model # SS-L1; Bonner Springs, KS) with a mix time of 5 min as described previously (Schumacher et al., 2017). Feed was discharged at a rate of approximately 4.5 kg/min into a bucket elevator (Universal Industries, Cedar Falls, IA) fitted with 74 buckets (114 cm^3 each), and then discharged through a 25.4-cm 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 2.5- and 5.0-kg batches using a mix time of 5.0 min. Validation of mixers prior to the experiment to achieve a coefficient of variation of less than 10% was done following previously described procedures (McCoy, 2005). The volume of rice hulls and feed added to

Table 1. Diet composition (as-fed basis)

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[1]	0.15
Sow add pack[2]	0.50
Vitamin premix[3]	0.25
Phytase[4]	0.02
Total	100
Calculated analysis, %[5]	
Crude protein	14.0
Crude fiber	2.2
Ether extract	4.0
Ca	0.85
P	0.62
Available P	0.46

[1]Each kilogram contains 26.4-g Mn, 110-g Fe, 110-g Zn, 11-g Cu, 198-mg I, and 198-mg Se.

[2] Each kilogram contains 110,000-mg choline, 44-mg biotin, 330-mg folic acid, and 990-mg pyridoxine.

[3] Each kilogram contains 4,400,000 IU vitamin A, 660,000 IU vitamin D3, 17,600 IU vitamin E, 1,760 mg menadione, 3,300 mg riboflavin, 11,000 mg pantothenic acid, 19,800 mg niacin, and 15.4 mg vitamin B12.

[4] HiPhos 2700, DSM Nutritional Products, Parsippany, NJ.

[5] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.

the mixing systems was designed to reflect the fill volume relative to mixer capacity of paddle mixers in a commercial setting.

Chemical Treatment

The procedures used, while reduced in scale compared with commercial production mills, attempt to replicate commercial conditions as closely as possible. Prior to initiation of the experiment, six 2.5-kg chemically treated rice hull batches were prepared using 2% MCFA blend (n = 2; 1:1:1 ratio of hexanoic, octanoic, and decanoic 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 = 3.25 kg/tonne). Untreated rice hulls (2.5 kg; 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 h at room temperature (21 °C) until initiation of experiment.

Prior to inoculation with PEDV, batches of feed were 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 1-step motion to dispose of feed into a waste container. A small quantity of residual feed remained in each mixer after this systematic priming and discharge procedure. Following priming of each laboratory scale mixer, a 2.5-kg batch of PEDV-negative feed was added to each mixer and mixed as described above. The mixer was then shut off, drive coupler removed from the drive unit motor, and a subsample was collected from 6 locations within each mixer for a total sample size of approximately 225 g. 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 laboratory scale mixer, the production scale system was primed, and negative sample collected. A 5-kg batch of PEDV-negative feed was added to the production scale mixer, allowed to mix for approximately 15 s, 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 50-kg batch of PEDV-negative feed was then added to the production scale mixer, mixed for 5.0 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

PEDV isolation, propagation, and titration were performed as described elsewhere (Chen et al., 2014). The viral inoculum was cell culture derived (USA/IN/2013/19338, passage 9) and had an initial concentration of 4×10^6 $TCID_{50}$/mL. This isolate has been previously shown to be pathogenic in young pigs (Thomas et al., 2015). A 1:10 dilution was performed using PBS to create 2,500 mL of 10^5 $TCID_{50}$/mL viral inoculum. Inoculation of the feed used similar procedures as those described by Schumacher et al. (2016b) and Cochrane et al. (2017). Briefly in this experiment, inoculation of feed to be used in each of the laboratory scale mixers was performed in 5-kg batches using an additional laboratory scale mixer in which 4.5 kg of PEDV-negative feed was added to the mixer and

500 mL of 10^5 $TCID_{50}$/mL–diluted viral inoculum was added to create 5 kg of 10^4 $TCID_{50}$/g–inoculated feed. This batch was mixed for 5 min, at which point it was split into 2 samples using a riffle splitter and weighed into 2.5-kg batches, bagged, and stored in a freezer (−12 °C) until inoculated into an appropriate laboratory scale mixer. This process was repeated 3 additional times, to create a total of eight 2.5-kg 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 5.0 min, at which point a sample of PEDV-inoculated feed was collected from 6 locations within the mixer. Inoculated feed was then discarded into biohazard waste bags using a complete inversion of the mixer following systematic procedure as described above with no tapping or additional cleaning action. The appropriate flush batch was added to the mixer and mixed for 5.0 min. A sample of the rice hull flush was collected from 6 locations within the mixer as described previously. The remaining flush was then discarded, and a subsequent 2.5-kg 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 8 laboratory-scale mixers with 2 replicates of each of the 4 chemical treatments (untreated rice hulls, formaldehyde-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 4.5-kg batch of PEDV-negative feed was added to a clean laboratory scale paddle mixer and 500 mL of 10^6 $TCID_{50}$/mL inoculum was slowly added to create a 5-kg batch of PEDV-inoculated feed (10^5 $TCID_{50}$/g). Upon conclusion of the addition of the virus, the batch was mixed for 5.0 min to ensure an even mix of virus into the feed inoculum. The PEDV feed inoculum was then added to 45 kg of PEDV-free swine diet in the production scale mixer to create the 50-kg batch of PEDV-positive feed (10^4 $TCID_{50}$/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 $TCID_{50}$/g) from laboratory scale mixer to create a single PEDV-positive sample. After inoculation of the production scale mixer, 36 kg of ground rice hulls was added directly to the mixer, along with 4 kg of MCFA (1:1:1 ratio of hexanoic, octanoic, and decanoic acid) to create a 10% MCFA rice hull flush with a similar mixer fill volume as a 50-kg batch of feed. After a 5.0-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 50-kg batch of PEDV-negative feed was then added to the production-scale mixer and allowed to mix for 5.0 min. A 225-g sample was collected from the mixer and remaining feed was discharged into the bucket elevator and collected at the bucket elevator discharge spout. Again, a 225-g sample was collected from 6 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 $TCID_{50}$/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 from mixing of 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 Kansas State University Molecular Diagnostic Research and Development Laboratory, three 50.0-g subsamples of feed from each collection point were 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 s, at which point they were allowed to settle overnight at 4 °C. On the next day, supernatant was collected, and aliquots prepared for further analysis. A total of 4 aliquots from each sample bottle were collected and stored at −20 °C until qRT-PCR analysis was performed within 7 d of inoculation on 1 aliquot per sample bottle. The remaining 3 samples per bottle were stored at −80 °C 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 processed 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 −80 °C 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 as described previously (Schumacher et al., 2016b, 2017). Reported values represent threshold cycle time (Ct) at which virus was detected. A greater Ct value indicates that more cycles must proceed until viral genetic material was detected, thus representing lower quantities of genetic material in the original sample.

Bioassay

Bioassay procedures used were the same as those described previously (Schumacher et al, 2016b; Cochrane et al., 2017). Bioassay samples were selected after qRT-PCR analysis included a composite positive and negative control from laboratory and production-scale mixers, rice hull flush samples from the untreated, formaldehyde, and 2% MCFA flushes of the laboratory-scale mixers, as well as subsequent feed for all 4 laboratory-scale treatments (Figure 1). Bioassay samples from the production-scale system included 10% MCFA rice hull flush and subsequent feed both collected from the discharge spout of the bucket elevator. Dust samples included those collected from mixing surfaces after manufacture of 10^4 $TCID_{50}$/g–inoculated swine feed, after the 10% MCFA rice hull flush, and subsequent feed after the 10% MCFA rice hull flush. Supernatant samples were allowed to thaw prior to inoculation at room temperature, beginning approximately 3 h prior to inoculation. Dust samples were prepared by combining the 3 positive control dust samples into a single, homogenous positive control dust sample. A total of 3, homogenous, dust samples (positive, 10% MCFA rice hull flush, 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 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. Upon arrival, piglets were ear tagged, weighed, and randomly assigned to bioassay treatment rooms. Fecal swabs were negative for PEDV, PDCoV, and transmissible gastroenteritis virus (**TGEV**) using qRT-PCR analysis. Serum was negative for PEDV antibody by an indirect fluorescent antibody (**IFA**) assay and negative for 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. Three pigs were housed per room with all pigs challenged with a single treatment. Each room had an independent ventilation system. Biosecurity protocols were in place to prevent viral spread between rooms. 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 inoculated on day 0 with 20 mL of the PBS supernatant by orogastric gavage. Each of 9 pigs (3 rooms) which were inoculated with dust samples followed similar procedures; however, the remaining solid fraction of the inoculum was placed in the mouth of each pig and were stimulated to swallow. Rectal swabs were collected daily from all piglets and tested for PEDV RNA via qRT-PCR on 2-, 0-, 2-, 4-, 6-, and 7-day postinoculation (dpi). Cecal content was evaluated for the presence of PEDV genetic material via qRT-PCR at necropsy on day 7.

Statistical Analysis

Data were analyzed using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) to determine

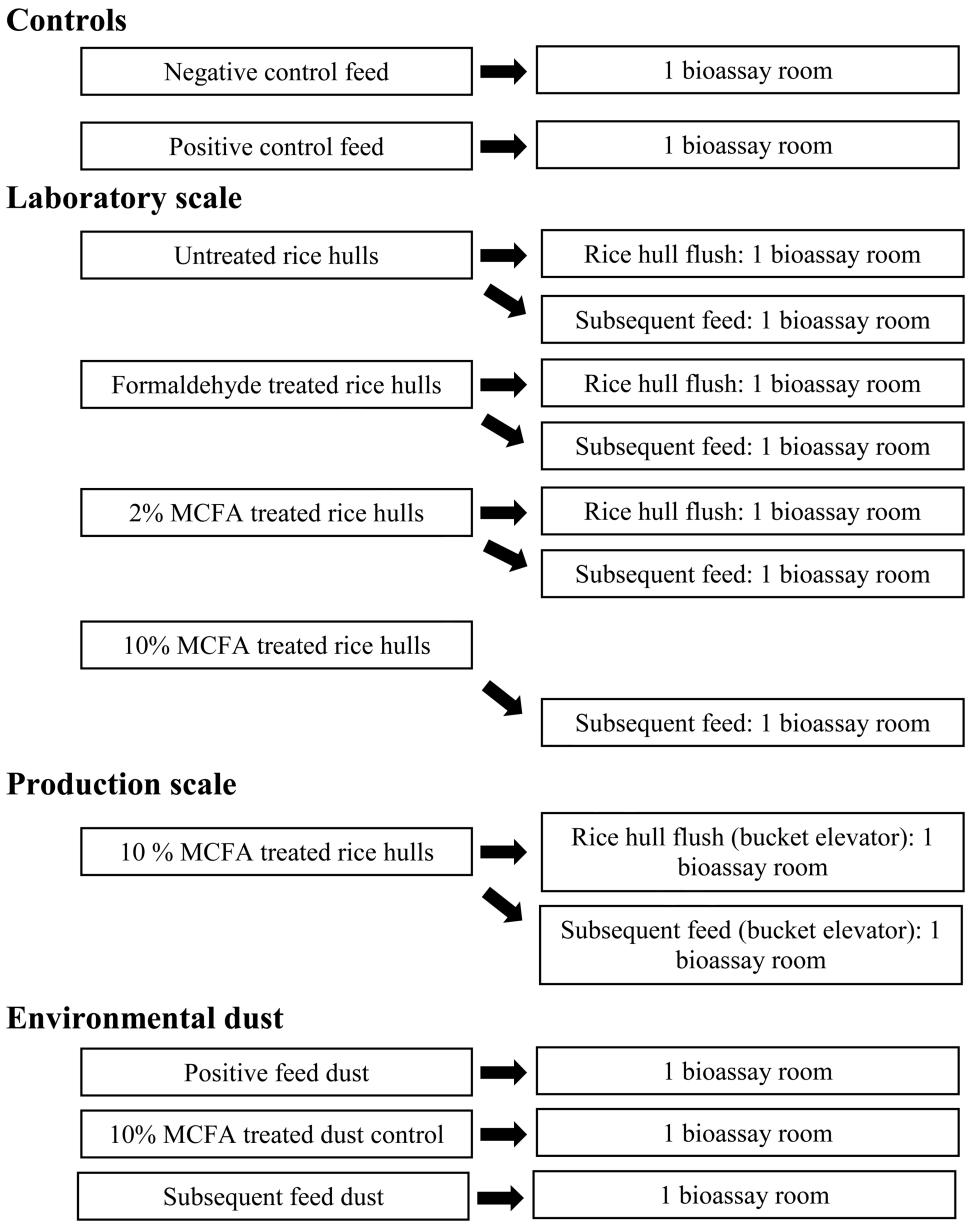

Figure 1. Experimental design distinguishing bioassay treatment selection. Laboratory scale mixers and production scale system were used to mix feed inoculated with porcine epidemic diarrhea virus (PEDV), flushed with appropriate rice hull flushes, and mixed a subsequent batch of feed. Medium-chain fatty acid (MCFA) was added on a wt:wt basis. One bioassay room represents a total of 3 pigs.

differences between the treatments. Pairwise comparisons were used to determine differences among flush strategies, with the model protected by the overall *F*-test. A cycle time value of 45 was used in the statistical analysis for samples not containing detectible genetic material. Results for response criteria were considered significant at $P \leq 0.05$.

RESULTS

Viral RNA Quantification

After qRT-PCR analysis, the composite negative feed sample did not have detectible RNA, and composite positive control feed sample contained detectible PEDV genetic material (Ct = 30.2, Table 2). Following a PEDV-positive batch of feed in laboratory scale mixers, 50% of the untreated rice hull flush samples had detectable PEDV RNA. The amount of detectible genetic material was less ($P < 0.05$) within the untreated rice hull flush sample compared with the PEDV-positive batch of feed. One of 6 formaldehyde-treated rice hull flush samples was positive for PEDV genetic material, and 2 out of 6 of the 2% MCFA rice hull samples had detectable PEDV RNA. In contrast, none of the 10% MCFA rice hull flush samples had detectible PEDV RNA. Chemically treated rice hull flushes using formaldehyde and 10% MCFA

reduced ($P < 0.05$) the quantity of detectible RNA present in the rice hull flush samples compared with the untreated rice hull flush. However, the 2% MCFA rice hull flush did not reduce ($P = 0.215$) the quantity of genetic material compared with the untreated rice hull flush. Importantly, no feed samples collected after an untreated or chemically treated rice hull flush had detectible PEDV genetic material. 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 detectible 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. Dust collected after mixing the positive feed had a large quantity of viral RNA (Table 3). 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% rice hull flush did not have detectible RNA.

Bioassay

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 all 3 positive control pigs. These 3 pigs had detectable PEDV RNA at all subsequent fecal sample collections as well as cecal contents at necropsy. No other flush feed bioassay pigs had detectible RNA in fecal swabs throughout the study or cecal content collected at necropsy. Pigs inoculated with the positive dust collected following mixing of inoculated feed were shedding PEDV by day 2 after oral inoculation

Table 2. Effect of chemically treated rice hull flushes on PEDV RNA detection and infectivity of samples collected in feed manufacturing equipment

	Rice hull treatment			
Item	Untreated	Formaldehyde[1]	2% MCFA[2]	10% MCFA
Prevalence, % positive (positive/total samples)				
Negative feed	0 (0/3)			
Positive feed	100 (3/3)			
Laboratory scale mixer				
Rice hull flush	50 (3/6)	17 (1/6)	33 (2/6)	0 (0/6)
Subsequent feed	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
Production scale mixer				
Rice hull flush	– – –	– – –	– – –	0 (0/3)
Subsequent feed	– – –	– – –	– – –	0 (0/3)
Production scale bucket elevator				
Rice hull flush	– – –	– – –	– – –	33 (1/3)
Subsequent feed	– – –	– – –	– – –	0 (0/3)
Cycle threshold, Ct				
Negative feed	45.0[a] (−)[3]			
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] (−)

Swine feed was inoculated with porcine epidemic diarrhea virus (PEDV) at a concentration of 10^4 $TCID_{50}$/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 2.5 kg with a mix time of 5 min.

[1]Sal CURB (Kemin Industries, Inc., Des Moines, IA) was added at a recommended level of 3.25 kg/ton.

[2]Medium chain fatty acid blend (1:1:1 ratio of hexanoic, octanoic, and decanoic acid) added on a wt:wt basis to ground rice hulls.

3(+) 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, whereas (−) indicates 0/3 pigs had detectible PEDV genetic material in fecal swabs throughout the full 7-d bioassay as well as did not have detectible PEDV genetic material in cecal contents at necropsy.

[a–c]Cycle threshold means lacking common superscript differ ($P < 0.05$). Pooled SEM = 0.85.

Table 3. Effect PEDV RNA detection and infectivity in environmental dust samples

		Fecal swabs				Cecum contents
Item	Inoculum, Ct[1]	−2 dpi	2 dpi	4 dpi	6 dpi	7 dpi
Positive feed dust	29.4	− − −	+ + +	+ + +	+ + +	+ + +
10% MCFA rice hull dust	33.7	− − −	− − −	− − −	− − −	− − −
Subsequent feed dust	45.0	− − −	− − −	− − −	− − −	− − −

Dust samples were collected from the laboratory and production mixers from nonfeed contact surfaces. Infectivity was evaluated in a 10-d-old pig bioassay with 3 pigs per dust type. Pigs were individually inoculated on 0 dpi. (+) indicates that an individual pig was found to have detectable PEDV genetic material in the respective sample using qRT-PCR. (−) indicates that an individual pig did not have detectable PEDV genetic material in the respective sample.

[1]Positive feed dust, average of $n = 3$, 10% MCFA rice hull dust, $n = 1$; subsequent feed dust, $n = 1$. PEDV qRT-PCR cycle threshold (Ct).

and continued to shed through necropsy at 7 dpi in both fecal samples and cecal content. However, pigs inoculated with the dust from the 10% MCFA rice hull flush batches or the subsequent feed batch did not have any PEDV genetic material detected in fecal samples throughout the bioassay or in cecal contents at necropsy.

DISCUSSION

Epidemiological investigation has indicated feed or feed ingredients associated with PEDV transmission (Bowman et al., 2015; Aubry et al., 2017). Furthermore, transmission through feed and feed ingredients has been demonstrated experimentally (Dee et. al, 2014; Pasick et al., 2014; Pillatzki et al., 2015). Efforts to characterize the minimum infectious dose using bioassay of PEDV in feed have shown that a low quantity of PEDV is required to contaminate feed (Schumacher et al., 2016b).

Dee et al. (2014) found that samples collected from the interior surface of feed bins have the ability to cause infection in naïve pigs. Previous work from our group has also shown that PEDV genetic material is widely dispersed within a feed manufacturing facility (Schumacher et al., 2017). When batches of feed are inoculated with biological agents, specifically PEDV, a large amount of dust is generated and rapidly fills the manufacturing area depositing on virtually every surface. We have presumed that this material is widely dispersed through viral particles carried on dust. In support of this presumption, the results in this study indicate that the presence of PEDV genetic material in dust can be infectious. To the best of our knowledge, this is the first data reported that has indicated dust within a feed mill can contain an infectious viral pathogen. Although the current experiment was performed in a controlled setting with equipment that is smaller scale than commercial production facilities, it serves as a proof of concept that should be further evaluated with additional research.

In the current experiment, when dust was collected following manufacturing PEDV-inoculated feed, a 10% MCFA-treated rice hull flush, and subsequent batch of feed, the only dust sample which caused infection was dust collected following manufacturing PEDV-inoculated feed. Due to the fact that dust samples were collected from the same location following the appropriate batch, it is reasonable to believe the dust collected at each time point was generated during manufacture of the specific batch alone and did not contain carryover material from previous batches. This demonstrates that dust generated during the 10% MCFA flush and subsequent batch of feed was not infectious. However, in commercial mills, dust would accumulate over additional batches and the nature of such dust accumulation was not evaluated during our study.

Current feed manufacturing processes such as grain and ingredient receiving, storage, feed manufacture, and final delivery to site of consumption have the potential to incorporate infectious material into the manufacturing system, ultimately leading to potentially infectious feed. Batch-to-batch feed manufacturing and equipment surface contamination has been demonstrated (Schumacher et al, 2016a; 2017). Also, these surfaces have been shown to be difficult to decontaminate (Huss et al., 2017). In such event that biosecurity measures fail and pathogens such as PEDV enter a feed manufacturing facility, methods to reduce risk of PEDV transmission by feed or ingredients shift to mitigation strategies. Multiple strategies have been proposed to reduce transmission, typically falling into point-in-time or residual duration of activity strategies. Point-in-time strategies include the use of irradiation and thermal processing, whereas residual duration of activity strategies is commonly thought of as chemical mitigation in which feed additives such as MCFA, formaldehyde, essential oils, and dietary acidifiers are included to reduce risk of disease transmission. Both strategies have

advantages and disadvantages, and incorporation into feed safety plans should be specific to a given set of circumstances.

In addition to temporary solutions following introduction of pathogens such as chemical mitigation of feed, the strategy must shift to elimination of the pathogen altogether from the facility. In a similar manner by which pharmaceutical compounds are flushed through feed manufacturing equipment (FDA, 1976), sequencing of feed has been proposed to potentially reduce subsequent cross-contamination of PEDV (Schumacher et al., 2016b). The quantity of detectible genetic material was reduced as subsequent batches of feed were manufactured. Also, genetic material was detectible for a longer duration in samples collected from the discharge spout of the experimental bucket elevator compared with samples collected from the mixer. Samples collected from the mixer caused infection for 2 subsequent batches of feed, whereas no samples collected from the bucket elevator discharge spout for batches of feed following the inoculated batch caused infection. Thus, it has been shown that contamination within feed manufacturing equipment can be reduced using protocols to minimize contamination in later batches of feed. In relation to the current study, a similar reduction in the amount of quantifiable genetic material was observed with increasing number of batches through the mixing and handling equipment. Increasing the number of batches through a system reduces the level of contamination within the feed and on feed contact surfaces. However, environmental contamination is still a significant concern. In the current study, the presence of detectible viral RNA in the 10% MCFA-treated rice hull flush sample collected from the bucket elevator discharge spout, whereas no genetic material was found when collected from the mixer suggests that bucket elevators can be a significant cross-contamination source within feed manufacturing systems. The inability of feed manufacturing equipment to be completely cleaned between batches of feed, specifically the boot of bucket elevators is a likely cross-contamination source.

To compliment the abrasive characteristics of rice hulls, it was hypothesized that chemical treatment of flush material would provide additional benefit beyond rice hulls alone. The use of chemical treatments to reduce PEDV quantity and infectivity characteristics within feed and feed ingredients has been extensive, including commercial feed additives (Trudeau et al., 2016) as well as MCFA's (Cochrane et al., 2015; 2017; Dee et al., 2016) and formaldehyde (Dee et al., 2015; Cochrane et al., 2017). The use of MCFA's has shown significant potential to provide substantial efficacy in reduction of PEDV transmission and could potentially be implemented in the future. Inclusion rates of MCFA's used in previous PEDV mitigation studies have used a 2% maximum inclusion on a wt:wt basis. In the current study, the 2% inclusion rate reduced the number of samples with detectible RNA compared with rice hulls alone, whereas no PEDV RNA was detected in the 10% MCFA inclusion. Thus, a gradient in efficacy was observed with greater efficacy as PEDV mitigation flushes with 10% inclusion of MCFA.

The use of commercial formaldehyde products, while efficacious as compounds to reduce disease transmission risk, is not applicable in all situations. Limitations exist such that use of commercial formaldehyde products is not practical in all feed manufacturing facilities due to the lack of application equipment or low usage in swine or poultry feed making justification of such a system impractical. In the current study, the inclusion rate was based on manufacturer recommendation for complete feed and was found to be more efficacious at reducing the amount in quantifiable genetic material in the flush samples than untreated rice hulls and rice hulls treated with 2% MCFA. This efficacy is consistent with previous literature demonstrating formaldehyde products are efficacious at reducing quantities of detectible genetic material as well as infectivity (Dee et al., 2015; Cochrane et al., 2017). Although no differences were observed in bioassay among treated and untreated rice hulls, chemical treatment of rice hull flushes including the use of formaldehyde and MCFA's reduced the quantity of detectible genetic material.

In conclusion, the dust collected after manufacturing PEDV-inoculated feed contains a large quantity of viral RNA and has the potential to serve as a vector for PEDV transmission. Also, the use of rice hull flushes effectively reduced the quantity of detectible RNA present after mixing a batch of PEDV-positive feed. Additionally, chemical treatment of rice hulls with formaldehyde and 10% MCFA provided additional reduction in detectible RNA and yielded no infectivity in naïve pigs. Such evidence demonstrates the potential for strategically timed flush steps with material such as chemically treated rice hulls to reduce contamination by pathogens within a feed manufacturing facility, providing a useful decontamination procedure in the event a feed manufacturing facility becomes compromised.

LITERATURE CITED

Aubry, P., J. L. Thompson, T. Pasma, M. C. Furness, and J. Tataryn. 2017. Weight of the evidence linking feed to an outbreak of porcine epidemic diarrhea in Canadian swine herds. J. Swine Health Prod. 25:69–72.

Bowman, A. S., R. A. Krogwold, T. Price, M. Davis, and S. J. Moeller. 2015. Investigating the introduction of porcine epidemic diarrhea virus into an Ohio swine operation. BMC Vet. Res. 11:38. doi:10.1186/s12917-015-0348-2

Chen, Q., G. Li, J. Stasko, J. T. Thomas, W. R. Stensland, and A. E. Pillatzki. 2014. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. J. Clin. Microbiol. 52. doi:10.1128/jcm.02820-13

Cochrane, R. A. 2015. Chemical mitigation of microbial pathogens in animal feed and ingredients. MS Diss. Kansas State Univ., Manhattan, KS.

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, et al. 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 (Suppl 2):50. doi:10.2527/msasas2016-107

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. Bia, et al. 2017. Effect of pelleting on survival of porcine epidemic diarrhea virus-contaminated feed. J. Anim. Sci. 95:1170–1178. doi:10.2527/jas.2016.0961

Cochrane, R. A., J. C. Woodworth, S. S. Dritz, A. R. Huss, C. R. Stark, R. A. Hesse, M. D. Tokach, J. F. Bai, and C. K. Jones. 2015. Evaluating chemical mitigation of porcine epidemic diarrhea virus in swine feed and ingredients. J. Anim. Sci. 93 (Suppl. 2):90 (Abstr.).

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 naive pigs following consumption via natural feeding behavior: proof of concept. BMC Vet. Res. 10. doi:10.1186/s12917-014-0176-9

Dee, S., C. Neill, T. Clement, A. Singrey, J. Christopher-Hennings, and E. Nelson. 2015. An evaluation of porcine epidemic diarrhea virus survival in individual feed ingredients in the presence or absence of a liquid antimicrobial. Porcine Health Manag. 1:9. doi:10.1186/s40813-015-0003-0

Dee, S., C. Neill, A. Singrey, T. Clement, R. Cochrane, C. Jones, G. Patterson, G. Spronk, J. Christopher-Hennings, and E. Nelson. 2016. Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus. BMC Vet. Res. 12:51. doi:10.1186/s12917-016-0674-z

FDA, Department of Health and Human Services. 1976. Current good manufacturing practice for medicated feeds. 21 C.F.R. part 225.65. Fed. Regist. 41:52618.

Huss, A. R., L. L. Schumacher, R. A. Cochrane, E. Poulsen, J. Bai, J. C. Woodworth, S. S. Dritz, C. R. Stark, and C. K. Jones. 2017. Elimination of porcine epidemic diarrhea virus in an animal feed manufacturing facility. PLoS One 12:e0169612. doi:10.1371/journal.pone.0169612

McCoy, R. A. 2005. Mixer testing. In: Schofield, E, editor, Feed manufacturing technology. Am. Feed Ind. Assoc., Arlington, VA. p. 620–622.

Muckey, M. B. 2016. Evaluation of surface sanitation to prevent biological hazards in animal food manufacturing. MS Diss. Kansas State Univ., Manhattan, KS.

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. Transbound. Emerg. Dis. 61:397–410. doi:10.1111/tbed.12269

Pillatzki, A. E., P. C. Gauger, D. M. Madson, E. R. Burrough, J. Zhang, Q. Chen, D. R. Magstadt, P. H. E. Arruda, G. W. Stevenson, and K. J. J. Yoon. 2015. Experimental inoculation of neonatal piglets with feed naturally contaminated with porcine epidemic diarrhea virus. J. Swine Health Prod. 23: 317–320.

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, S.S. Dritz, and M. D. Tokach. 2016a. Utilizing feed sequencing to decrease the risk of porcine epidemic diarrhea virus (PEDV) cross-contamination during feed manufacturing. J. Anim. Sci. 94 (Suppl. 2):163 (Abstr.)

Schumacher, L. L., A. R. Huss, R. A. Cochrane, C. R. Stark, J. C. Woodworth, J. Bai, E. G. Poulsen, Q. Chen, R. G. Main, J. Zhang, et al. 2017. Characterizing the rapid spread of porcine epidemic diarrhea virus (PEDV) through an animal food manufacturing facility. PLoS One 12:e0187309. doi:10.1371/journal.pone.0187309

Schumacher, L. L., J. C. Woodworth, C. K. Jones, Q. Chen, J. Zhang, P. C. Gauger, C. R. Stark, R. G. Main, R. A. Hesse, M. D. Tokach, et al. 2016b. Evaluation of the minimum infectious dose of porcine epidemic diarrhea virus in virus-inoculated feed. Am. J. Vet. Res. 77:1108–1113. doi:10.2460/ajvr.77.10.1108

Thomas, J. T., Q. Chen, P. C. Gauger, L. G. Giménez-Lirola, A. Sinha, K. M. Harmon, D. M. Madson, E. R. Burrough, D. R. Magstadt, H. M. Salzbrenner, et al. 2015. Effect of porcine epidemic diarrhea virus infectious doses on infection outcomes in naïve conventional neonatal and weaned pigs. PLoS One 10:e0139266. doi:10.1371/journal.pone.0139266

Trudeau, M. P., H. Verma, F. Sampedro, P. E. Urriola, G. C. Shurson, J. McKelvey, S. D. Pillai, and S. M. Goyal. 2016. Comparison of thermal and non-thermal processing of swine feed and the use of selected feed additives on inactivation of porcine epidemic diarrhea virus (PEDV). PLoS One 11:e0158128. doi:10.1371/journal.pone.0158128