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Effects of medium chain fatty acids as a mitigation or prevention strategy against porcine epidemic diarrhea virus in swine feed

Annie B. Lerner,[†] Roger A. Cochrane,[‡] Jordan T. Gebhardt,[†] Steve S. Dritz,[‖] Cassandra K. Jones,[†] Joel M. DeRouchey,[†] Mike D. Tokach,[†] Robert D. Goodband,[†,1] Jianfa Bai,[‖] Elizabeth Porter,[‖] Joe Anderson,[‖] Phillip C. Gauger,[$] Drew R. Magstadt,[$] Jianqiang Zhang,[$] Benjamin Bass,[¶] Theodore Karnezos,[¶] Brenda de Rodas,[¶] and Jason C. Woodworth[†]

[†]Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan, KS 66506-0201, [‡]Pipestone Grow Finish, Pipestone, MN, [‖]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506-0201, [$]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, [¶]PMI, Arden Hills, MN

[1]Corresponding author: goodband@ksu.edu

ORCiD numbers: 0000-0002-6144-6714 (J. T. Gebhardt); 0000-0001-6371-0729 (S. S. Dritz).

Abstract

Feed has been shown to be a vector for viral transmission. Four experiments were conducted to: 1) determine if medium chain fatty acids (**MCFA**) are effective mitigants when applied to feed both pre- and post-porcine epidemic diarrhea virus (**PEDV**) inoculation measured by quantitative reverse transcription polymerase chain reaction (**qRT-PCR**), 2) evaluate varying levels and combinations of MCFA measured by qRT-PCR, and 3) evaluate selected treatments in bioassay to determine infectivity. In exp. 1, treatments were arranged in a 2 × 2 + 1 factorial with main effects of treatment (0.3% commercial formaldehyde [**CF**] product, Sal CURB [Kemin Industries, Inc.; Des Moines, IA], or 1% MCFA blend (Blend) of 1:1:1 C6:C8:C10 [PMI, Arden Hills, MN]) and timing of application (pre- or post-inoculation with PEDV) plus a positive control (**PC**; feed inoculated with PEDV and no treatment). All combinations of treatment and timing decreased detectable PEDV compared with the PC ($P < 0.05$). Pre-inoculation treatment elicited decreased magnitude of PEDV detection (cycle threshold value) compared with post-inoculation ($P = 0.009$). Magnitude of PEDV detection was decreased for CF compared with Blend ($P < 0.0001$). In exp. 2, pre-inoculation treatments consisted of: 1) PC, 2) 0.3% CF, 3 to 5) 0.125% to 0.33% C6:0, 6 to 8) 0.125% to 0.33% C8:0, 9 to 11) 0.125% to 0.33% C10:0, and 12 to 15) 0.125% to 0.66% C5:0. Treating feed with 0.33% C8:0 resulted in decreased ($P < 0.05$) PEDV detection compared with all other treatments. Increasing concentration of each individual MCFA decreased PEDV detectability ($P < 0.042$). In exp. 3, pre-inoculation treatments consisted of: 1) PC, 2) 0.3% CF, 3 to 7) 0.25% to 1% Blend, 8 to 10) 0.125% to 0.33% C6:0 + C8:0, 11 to 13) 0.125% to 0.33% C6:0 + C10:0, and 14 to 16) 0.125% to 0.33% C8:0 + C10:0. Treating feed with CF, 0.5% Blend, 0.75% Blend, 1% Blend, all levels of C6:0+C8:0, 0.25% C6:0 + 0.25% C10:0, 0.33% C6:0 + 0.33% C10:0, 0.25% C8:0 + 0.25% C10:0, or 0.33% C8:0 + 0.33% C10:0 elicited decreased detection of PEDV compared with PC ($P < 0.05$). Increasing concentration of each MCFA combination decreased PEDV detectability (linear, $P < 0.012$). In exp. 4, feed was treated pre-inoculation with: 1) no treatment (PC), 2) 0.3% CF, 3) 0.5% Blend, or 4) 0.3% C8:0 and analyzed via

qRT-PCR and bioassay. Adding 0.5% Blend or 0.3% C8:0 resulted in decreased PEDV compared with PC and only PC resulted in a positive bioassay. Therefore, MCFA can decrease detection of PEDV in feed. Further, inclusion of lower levels of MCFA than previously evaluated are effective against PEDV.

Key words: medium chain fatty acid, porcine epidemic diarrhea virus, swine

Abbreviations

CF	commercial formaldehyde
Ct	cycle threshold
dpi	day(s) post-inoculation
MCFA	medium chain fatty acid(s)
PBS	phosphate-buffered saline
PC	positive control
PCR	polymerase chain reaction
PEDV	porcine epidemic diarrhea virus
qRT-PCR	quantitative reverse transcription polymerase chain reaction

Introduction

The introduction of porcine epidemic diarrhea virus (**PEDV**) to the U. S. swine herd prompted significant investigation regarding routes of viral transmission. It was validated in both controlled experiments (Dee et al., 2014a; Pasick et al., 2014; Schumacher et al., 2016) and epidemiological studies (Bowman et al., 2015; Aubry et al., 2017) that feed ingredients and complete feed may serve as a vehicle for viral transmission. Thus, feed additives have been explored to reduce or prevent viral transmission in swine feed. Medium chain fatty acids (**MCFA**), which consist of 6 to 12 carbon atoms, have emerged as a promising technology to disrupt virus activity within feed, potentially due to interaction with the viral membrane, preventing viral replication (Thormar et al., 1987; Cochrane, 2018). Cochrane et al. (2020) demonstrated the efficacy of MCFA as an effective strategy to decrease detectable genetic material and infectivity in complete swine feed. Adding 1% MCFA blend containing hexanoic (C6:0), octanoic (C8:0), and decanoic (C10:0) acids in a 1:1:1 ratio significantly reduced PEDV detection in swine feed when applied prior to inoculation (Cochrane et al., 2019). Gebhardt et al. (2020) also observed a decrease in detectable virus when the feed was manufactured with MCFA and stored for 40 d before inoculation with PEDV. However, there is no information to determine if application of MCFA pre- or post-inoculation is equally effective in reducing the viral activity in feed. Further, varying combinations of MCFA and lower inclusion rates that may be more economical have not been thoroughly evaluated. Therefore, the objectives of this set of experiments were to determine: 1) the effects of timing of MCFA application, 2) the impact of varying combinations of different fatty acids and inclusion levels, and 3) the effects of selected MCFA treatments in a bioassay.

Materials and Methods

Chemical treatments

Chemical treatments included in exp. 1 were 0.3% commercial formaldehyde (**CF**)-based product (Sal CURB; Kemin Industries, Inc.; Des Moines, IA) and 1% MCFA blend (1:1:1 ratio of C6:0, C8:0, and C10:0, PMI Nutritional Products, Arden Hills, MN) applied either pre- or post-inoculation with PEDV. In all experiments, pre-inoculation chemical treatments occurred 24 h prior to PEDV inoculation. Post-inoculation chemical treatments were applied within 1 h of virus addition then shaken to ensure even dispersion and stored overnight. There were six replications (250 mL bottles) per treatment.

Chemical treatments (administered prior to viral inoculation) included in exp. 2 were: 1) non-treated, PEDV inoculated control (positive control [**PC**]), 2) 0.3% CF (Sal CURB; Kemin Industries; Des Moines, IA), 3) 0.125% C6:0, 4) 0.25% C6:0, 5) 0.33% C6:0, 6) 0.125% C8:0, 7) 0.25% C8:0, 8) 0.33% C8:0, 9) 0.125% C10:0, 10) 0.25% C10:0, 11) 0.33% C10:0, 12) 0.125% C5:0, 13) 0.25% C5:0, 14) 0.33% C5:0, and 15) 0.66% C5:0. There were four replications per treatment.

Chemical treatments (administered prior to viral inoculation) included in exp. 3 were: 1) PC, 2) CF-based product (Sal CURB; Kemin Industries; Des Moines, IA), 3) 0.25% MCFA blend (1:1:1 ratio of C6:C8:C10), 4) 0.375% MCFA blend, 5) 0.500% MCFA blend, 6) 0.750% MCFA blend, 7) 1.0% MCFA blend, 8) 0.125% C6:0 + 0.125% C8:0, 9) 0.25% C6:0 + 0.25% C8:0, 10) 0.33% C6:0 + 0.33% C8:0, 11) 0.125% C6:0 + 0.125% C10:0, 12) 0.25% C6:0 + 0.25% C10:0, 13) 0.33% C6:0 + 0.33% C10:0, 14) 0.125% C8:0 + 0.125% C10:0, and 15) 0.25% C8:0 + 0.25% C10:0. There were four replications per treatment.

Treatments for exp. 4 included: 1) PC, 2) 0.3% CF (Sal CURB; Kemin Industries; Des Moines, IA), 3) 0.5% MCFA blend (1:1:1 ratio of C6:C8:C10), and 4) 0.3% C8. There were three replications per treatment.

Feed preparation and chemical application

A complete swine diet (corn- and soybean meal-based) was manufactured at the O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. A new batch of feed was manufactured for each experiment and did not contain specialty ingredients (whey, further processed soybean meal, animal plasma protein, or fish products) or antibiotics. Pre-inoculation chemical treatments were applied to 100 g of feed which was then mixed for 15 min using a mason jar feed mixer (Central Machine Shop, Purdue University, West Lafayette, IN) with 10 hex nuts to ensure agitation. Then, 22.5 g of treated feed was placed in a polyethylene bottle (250 mL Nalgene, square wide-mouth high-density polyethylene; Thermo Fisher Scientific, Waltham, MA) and stored at ambient temperature for 24 h.

Post-inoculation chemical treatment (exp. 1 only) occurred for each replication in the 250 mL bottle. Treatment was added within 1 h of inoculation and immediately shaken to ensure dispersion, then stored at ambient temperature for 24 h.

PEDV isolate and inoculation

The U.S. PEDV prototype strain cell culture isolate USA/IN19338/2013, passage 9 (PEDV19338) was used to inoculate feed. Virus isolation, propagation, and titration were performed in Vero cells (ATCC CCL-81) as described by Chen et al. (2014). The stock virus contained an initial concentration of 10^5 $TCID_{50}$/mL.

Inoculation was performed at the Kansas State University College of Veterinary Medicine Virology Laboratory (exps. 1, 2, and 3) and Iowa State University (exp. 4). All treatments were inoculated using an appropriately sized pipet to ensure even distribution of virus within the feed matrix. Each bottle received 2.5 mL of diluted viral inoculum, resulting in a final PEDV concentration of 10^4 $TCID_{50}$/g of feed. The pretreatment bottles received viral inoculation 24 h after chemical treatment, whereas the post-inoculation chemical treatments were applied within 1 h of viral inoculation. Bottles were then shaken for 15 s to further distribute virus throughout the feed.

Real-time PCR analysis

Bottles were stored at ambient temperature and 100 mL of phosphate-buffered saline (**PBS**; pH 7.4, Life Technologies, Grand Island, NY) was placed in each bottle containing 22.5 g of inoculated feed at 24-h post inoculation. Samples were swirled to ensure even mixing and stored at 4 °C for 24 h at which point supernatant was collected and stored at –80 °C until quantitative reverse transcription polymerase chain reaction (**qRT-PCR**) or bioassay was performed.

Quantitative real-time reverse transcription PCR procedures were conducted as previously described in the study of Gebhardt et al. (2019); 50 µL of supernatant from each sample was loaded into a deep well plate and extracted using a Kingfisher 96 magnetic particle processor (Fisher Scientific, Pittsburgh, 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 µL. One negative extraction control consisting of all reagents except the sample was included in each extraction. The extracted RNA was frozen at –20 °C until assayed by qRT-PCR. Analyzed values indicate cycle threshold (**Ct**) where virus was detected. Lower values indicate a greater magnitude of nucleic acid detection, but not necessarily infectivity.

Bioassay (experiment 4)

The bioassay procedure was carried out using the same procedures and same pig source used in previously reported studies (Schumacher et al., 2016, 2018; Gebhardt et al., 2019). The Iowa State University Institutional Animal Care and Use Committee reviewed and approved the pig bioassay protocol (IACUC #18-390). Fifteen, mixed-sex, commercial pigs (10 d of age) were obtained from a sow herd with no prior exposure to PEDV. Pigs were confirmed to be negative for PEDV, porcine delta coronavirus, and transmissible gastroenteritis virus based on fecal swab analysis upon arrival. To further confirm PEDV negative status, blood serum was analyzed for PEDV antibodies by an indirect fluorescent antibody assay. All assays were conducted at the Iowa State University Veterinary Diagnostic Lab. Pigs were allowed 2 d of adjustment prior to the bioassay. All pigs were housed individually with three pigs serving as the negative control without viral challenge and three pigs per treatment for the PC, 0.3% CF, 0.5% MCFA blend, and 0.3% C8:0 treatments. During the bioassay, rectal swabs were collected on days –2, 0, 3, 5, and 7 post-inoculation (**dpi**) from all pigs and tested for PEDV RNA via qRT-PCR. Following humane euthanasia at 7 dpi, cecal contents were collected and tested for PEDV RNA via qRT-PCR.

Statistical analysis

In all experiments, each 250 mL bottle was considered a replicate experimental unit and data were analyzed using PROC GLIMMIX in SAS (SAS Institute 9.4, Inc. Cary, NC). In exp.1, qRT-PCR data were analyzed for the fixed effects of chemical treatment or time of application. In exp. 2 through 4, the fixed effect of pre-inoculation treatment was evaluated. In exp. 2 and exp. 3, linear and quadratic responses were also evaluated with increasing doses of individual or combination MCFA. These linear and quadratic contracts included the PC and coefficients were generated using PROC IML to account for unevenly spaced inclusion levels. Results were considered significant at $P < 0.05$ and marginally significant at $P > 0.05$ and $P < 0.10$.

Results

Experiment 1

There was no evidence of an interaction between the timing of chemical application and chemical mitigant ($P = 0.326$; Table 1). Treating feed prior to PEDV inoculation resulted in decreased ($P = 0.009$) PEDV detection compared with feed treated with chemicals after PEDV inoculation. Also, regardless of the time of application, treating feed with a formaldehyde-based product resulted in decreased ($P < 0.001$) PEDV detection compared with MCFA-treated feed (Table 1). All four chemical treatments resulted in decreased ($P < 0.05$) PEDV detection compared with the PC.

Experiment 2

There was a significant effect ($P < 0.001$) of treatment (applied pre-inoculation) on the detectable PEDV (Table 2). Feed treatment

Table 1. Effect of chemical and timing of application in relation to PEDV inoculation on PEDV detection using qRT-PCR (exp. 1)[1]

		Pre-inoculation		Post-inoculation					
Item	PC	MCFA	Formaldehyde-based product	MCFA	Formaldehyde-based product	SEM	Timing × Chemical, P-value <	Timing, P-value <	Chemical, P-value <
qrt-PCR, Ct[2]	26.5[d]	30.6[b]	32.4[a]	28.8[c]	31.5[a,b]	0.46	0.326	0.009	0.001

[1]A total of 30 samples (6 samples per treatment) were used. An initial tissue culture (2.5 mL diluted PEDV inoculum, 10^5 $TCID_{50}$/mL) was added to 22.5 g of swine diet treated with either an MCFA blend or CF. PC = non-chemically treated feed inoculated with PEDV. MCFA treatment consisted of a 1:1:1 blend of C6:C8:C10 (hexanoic, octanoic, and decanoic acids, respectively; PMI, Arden Hills, MN) applied to swine feed at an addition of 1%. CF-based product (Sal CURB; Kemin Industries, Inc.; Des Moines, IA) was applied at 0.3%. Pre-inoculation indicates that the chemical treatments were applied before inoculation with PEDV. Post-inoculation indicates that chemical treatments were applied after inoculation with PEDV.
[2]Ct required to detect viral genetic material. A high Ct value indicates less genetic material present.
[a–d]Means with differing superscripts differ $P < 0.05$.

Table 2. Effect of treating swine feed with increasing levels of individual MCFA on PEDV detection using qRT-PCR (exp. 2)[1]

Item	qRT-PCR, Ct[2]	SEM	
PC	27.2[g]	0.35	
Formaldehyde-based product	29.3[b]		
C6:0			
0.125%	27.8[defg]	Linear, *P* =	0.001
0.25%	28.9[bc]	Quadratic, *P* =	0.831
0.33%	29.4[b]		
C8:0			
0.125%	28.8[bcd]	Linear, *P* =	0.001
0.25%	29.0[bc]	Quadratic, *P* =	0.263
0.33%	31.3[a]		
C10:0			
0.125%	27.7[efg]	Linear, *P* =	0.146
0.25%	28.4[bced]	Quadratic, *P* =	0.042
0.33%	27.4[fg]		
C5:0			
0.125%	27.1[g]	Linear, *P* =	0.001
0.25%	27.2[fg]	Quadratic, *P* =	0.578
0.33%	27.3[fg]		
0.66%	28.3[cdef]		

[1]A total of 60 samples (4 per treatment) were used. An initial tissue culture (2.5 mL diluted PEDV inoculum, 10^5 $TCID_{50}$/mL) was added to 22.5 g of swine diet treated with either CF or individual levels of C6:0, C8:0, C10:0, or C5:0 (PMI, Arden Hills, MN). PC = non-chemically treated feed inoculated with PEDV. CF-based product (Sal CURB; Kemin Industries, Inc.; Des Moines, IA) was applied at 0.3%.
[2]Ct required to detect viral genetic material. A high Ct value indicates less genetic material present.
[a–g]Means with differing superscripts differ (*P* < 0.05).

Table 3. Effect of treating swine feed with increasing levels of MCFA combinations on PEDV detection using qRT-PCR (exp. 3)[1]

Item	qRT-PCR, Ct[2]	SEM	
PC	27.8[f]	0.72	
Formaldehyde-based product	32.7[ab]		
MCFA Blend, %			
0.250	29.7[def]	Linear, *P* =	0.001
0.375	29.4[def]	Quadratic, *P* =	0.347
0.500	32.3[abc]		
0.750	31.8[abc]		
1.000	33.2[a]		
C6:0 + C8:0, %			
0.125[3]	30.7[bcde]	Linear, *P* =	0.001
0.25	31.4[abcd]	Quadratic, *P* =	0.291
0.33	32.7[ab]		
C6:0 + C10:0, %			
0.125	29.3[ef]	Linear, *P* =	0.001
0.25	30.4[cde]	Quadratic, *P* =	0.648
0.33	30.9[bcde]		
C8:0 + C10:0, %			
0.125	29.4[ef]	Linear, *P* =	0.012
0.25	31.3[abcde]	Quadratic, *P* =	0.237
0.33	30.3[cde]		

[1]A total of 64 samples (4 per treatment) were used. An initial tissue culture (2.5 mL diluted PEDV inoculum, 10^5 $TCID_{50}$/mL) was added to 22.5 g of swine diet treated with either CF, 1:1:1 MCFA blend of (C6:C8:C10, respectively), or combinations of C6:0, C8:0, C10:0. (PMI, Arden Hills, MN). PC = non-chemically treated feed inoculated with PEDV. MCFA blend consisted of a 1:1:1 blend of C6:C8:C10 (hexanoic, octanoic, and decanoic acids, respectively; PMI, Arden Hills, MN). CF-based product (Sal CURB; Kemin Industries, Inc.; Des Moines, IA) was applied at 0.3%.
[2]Ct required to detect viral genetic material. A higher Ct value indicates less genetic material present.
[3]Percentages listed indicate the level at which each MCFA was added to the feed.
[a–f]Means with differing superscripts differ (*P* < 0.05).

with 0.33% C8:0 resulted in decreased (*P* < 0.05) detectable PEDV compared with all other levels of MCFA, the formaldehyde-based product, and the PC. Alternatively, formaldehyde-based product, 0.25% C6:0, 0.33% C6:0, all levels of C8:0, 0.25% C10:0, 0.33% C10:0, and 0.66% C5:0 had decreased magnitude of viral nucleic acid detection compared with PC feed (*P* < 0.05). Further, increasing C6:0 and C8:0 addition from 0.125% to 0.33% resulted in decreased (linear, *P* < 0.001) PEDV detection. Increasing C10:0 addition resulted in a quadratic decrease in PEDV detection (*P* < 0.042). Lastly, increasing C5:0 from 0.125% and 0.66% resulted in linear decreases in viral detection (*P* = 0.001).

Experiment 3

When evaluating MCFA in combination and at varying concentrations applied pre-inoculation, there was a significant effect of treatment (*P* < 0.001; Table 3). Treatments that had significantly decreased (*P* < 0.05) PEDV detection values compared with the PC feed included: formaldehyde-based product, 0.50% Blend, 0.75% Blend, 1.0% Blend, all levels of C6:0 + C8:0, 0.25% C6:0 + 0.25% C10:0, 0.33% C6:0 + 0.33% C10:0, 0.25% C8:0 + 0.25% C10:0, and 0.33% C8:0 + 0.33% C10:0. Increasing MCFA blend resulted in decreased (linear, *P* = 0.001) viral nucleic acid detection. Increasing combination of C6:0 + C8:0, C6:0 + C10:0, and C8:0 + C10:0 from 0.25% to 0.66% resulted in a significant decrease in PEDV detection (linear, *P* < 0.012).

Experiment 4

The qRT-PCR results demonstrated a significant effect of pre-inoculation chemical treatment on feed (*P* < 0.001; Table 4), with 0.5% MCFA blend and 0.3% C8:0 having increased (*P* < 0.05) Ct compared with the PC and formaldehyde-based product treatments. For the bioassay, as expected, pigs inoculated with supernatant from negative control did not have positive PEDV bioassay results. Pigs inoculated with PC feed resulted in PEDV infection. For all other treatments, there was no evidence of PEDV infection detected for fecal swabs and cecal contents.

Discussion

The introduction of PEDV to North American swine herds in 2013 prompted significant research efforts to determine the viral route of transmission. Since then, literature has established that PEDV can be transmitted via feed ingredients and complete feed (Dee et al., 2014a, 2015; Schumacher et al., 2016). Additionally, the minimum infectious dose of PEDV in complete feed may be as low as 5.6×10^1 $TCID_{50}$/g (Schumacher et al., 2016). Given the small amount of virus needed to naturally infect pigs and the high volume of vehicle traffic at many feed manufacturing facilities, it is important to understand viral transmission within feed and feed mills. Equipment surfaces can retain PEDV RNA, and dust containing viral particles has been confirmed infectious in vivo (Huss et al., 2017; Gebhardt et al., 2018). Further, virus has been detected on the interior of feed delivery vehicles in a swine production system (Greiner, 2016). Thus, several strategies have been evaluated to control or mitigate the spread of PEDV in

Table 4. Effect of chemical mitigant used to treat swine feed on PEDV detection and infectivity using qRT-PCR and bioassay (exp. 4)[1]

		Fecal swabs					
Item	Feed Ct[2]	–2 dpi	0 dpi	3 dpi	5dpi	7 dpi	Cecal content, 7 dpi
Negative control	>36	---[3]	---	---	---	---	>36
PC	28.0[b]	---	---	+--	++-	+--	25.4[4]
Formaldehyde-based product	29.2[b]	---	---	---	---	---	>36
0.5% MCFA Blend	32.2[a]	---	---	---	---	---	>36
0.3% C8	32.9[a]	---	---	---	---	---	>36

[1]Each treatment was inoculated with the 10^5 $TCID_{50}$/mL PEDV resulting in 10^4 $TCID_{50}$/g PEDV inoculated feed matrix. The PEDV was diluted using PBS and supernatant collected evaluated for infectivity using a 12-d-old pig bioassay in three pigs per treatment (10 mL per pig). PC = non-chemically treated feed inoculated with PEDV. CF-based product (Sal CURB; Kemin Industries, Inc.; Des Moines, IA) was applied at 0.3%. MCFA blend consisted of a 1:1:1 blend of C6:C8:C10 (hexanoic, octanoic, and decanoic acids, respectively; PMI Arden Hills, MN) applied to the feed at a 0.5%.
[2]A Ct > 36 was considered no evidence of PEDV RNA.
[3]A (+) indicates evidence of PEDV infectivity and (-) indicates no evidence of infectivity with one symbol per pig.
[4]One pig had cecal contents that resulted in 25.4 Ct, while the other two pigs had no evidence of PEDV (Ct > 36) in cecal contents.
[a,b]Means with differing superscripts within column differ ($P < 0.05$).

feed manufacturing facilities and supply chains. Point-in-time processes such as pelleting (Cochrane et al., 2017) or irradiation (Trudeau et al., 2016) may be effective in decreasing detectable genetic material or infectivity, but do not provide lasting protection against potential recontamination. Equipment sanitation can be effective but is difficult to implement in high volume feed mills (Muckey, 2016). Therefore, feed additives remain a promising strategy to provide long-term protection from contaminated feed, though it is unclear whether treatment should occur before or after viral inoculation.

These are the first data to compare the effects of treating swine feed with mitigants (1% MCFA blend or 0.3% CF) either prior to or post-viral inoculation. The majority of literature evaluating feed mitigants incorporates the chemicals prior to viral inoculation (Dee et al., 2014b; Trudeau et al., 2016; Gebhardt et al., 2019). Efficacy of MCFA or formaldehyde to degrade viral RNA in feed has been demonstrated when feed is treated immediately before inoculation (Cochrane, 2018) and up to 40 d before inoculation (Gebhardt et al., 2020). It appears from our data that chemical treatments before or after inoculation will reduce the amount of detectable viral material compared with non-treated feed, yet pre-inoculation treatment increased Ct values beyond those of post-inoculation, though the magnitude of difference was marginal at approximately 1.3 Ct. These results are promising due to the fact that contamination can occur at many points in the ingredient procurement, feed manufacturing, and feed delivery process. Some ingredients (blood products) are a high risk for contamination due to being sourced from livestock processing facilities and may have greater affinity to retain PEDV viral activity over a period of time (Dee et al., 2016; Cochrane et al., 2018). However, contamination post-manufacturing is possible via infected equipment or contact surfaces (Schumacher et al., 2017).

Based on evidence that formaldehyde has antimicrobial characteristics (Wales et al., 2013), formaldehyde emerged as a potential PEDV mitigant after the U.S. outbreak. The application of Sal CURB (which is a combination of propionic acid and 37% aqueous formaldehyde) has been demonstrated to decrease the amount of detectable PEDV compared with infected, untreated feed as well as result in negative bioassay (Dee et al., 2014b; Cochrane et al., 2015). Our PCR and bioassay data support these findings that this source of CF effectively reduces the magnitude of detectable virus and prevents infection when tested in vivo.

Several experiments reported that while CF provides a notable decrease in detectable viral RNA, a 2% MCFA blend (1:1:1 blend of hexanoic, octanoic, and decanoic acids) also reduced quantifiable PEDV RNA compared with untreated controls (Cochrane, 2015, 2018). However, the use of formaldehyde may require specialized equipment and enhanced safety measures. Thus, other additives have been evaluated, such as organic acids, essential oils, and MCFA (Reichling et al., 2009; Cochrane et al., 2015; Trudeau et al., 2016; Gebhardt et al., 2019). After these findings, low inclusion levels were explored, and the addition of a 1% MCFA blend was found to be as effective as CF with a bioassay (Cochrane, 2018). Further exploration into individual MCFA showed that application of 0.66% C6:0, C8:0, or C10:0 also resulted in no evidence of PEDV infectivity in bioassays (Cochrane, 2018). The proposed mode of action for this phenomenon is thought to be the disruption of the viral envelope (Thormar et al., 1987; Cochrane, 2018). It is hypothesized that MCFA interact with the lipid bilayer of the envelope to prevent virus attachment to host cells and, ultimately, inhibit viral replication (Cochrane et al., 2018). In addition to PEDV, the use of MCFA has also been shown to result in the inactivation of other enveloped viruses, including vesicular stomatitis virus, herpes simplex virus, visna virus, respiratory syncytial virus, parainfluenza type 2, and avian influenza virus (Thormar et al., 1987; Hilmarsson et al., 2007; Hariastuti, 2011).

The qRT-PCR data in the present experiment are the first of our knowledge to explore MCFA at low inclusion levels (<0.66%) and combinations in an attempt to determine which, if any, MCFA may be delivering more antiviral activity than others. Our data show that at least 0.25% C6:0, all levels of C8:0, 0.25% C10:0 only, and 0.66% C5:0 resulted in decreased PEDV Ct values compared with the PC. Further, 0.5% or greater of the MCFA blend, all levels of C6:0 + C8:0 combinations, 0.25% C6:0 + 0.25% C10:0 or greater, and 0.25% C8:0 + 0.25% C10:0 or greater resulted in greater reduction of detectable PEDV compared with the PC. Evaluating the data from exp. 2 and exp. 3 together, it appears that C6:0 and C8:0 are providing the majority of the antiviral activity. Further research is needed to understand why certain MCFA are more efficacious at degrading virus, but the authors hypothesize that carbon chain length may impact the way the fatty acid interacts with viral membrane.

Thus, the 0.5% MCFA blend and 0.3% C8:0 were selected for evaluation in a bioassay. The lowest concentrations evaluated to our knowledge of MCFA blend (C6:C8:C10) or individual MCFA were 1% Blend and 0.66% C6:0, C8:0, or C:10 (Cochrane et al., 2018).

In the current experiment, all chemical treatments and the negative control resulted in no evidence of infectivity via bioassay with feed Ct values ranging from 29.2 to greater than 36. The PC treatment was the only treatment that resulted in evidence of infectivity via bioassay. Cochrane (2018) treated feed with 0.66% C8:0 and also prevented infection in a bioassay. In an experiment by Gebhardt et al. (2020), feed was treated with 0.5% C8 and inoculated 40 d after diet manufacturing, and the reduction in PEDV detection in feed was about 3 Ct. Though this was not fed to pigs in bioassay, this is similar to the present findings as 0.3% C8 increased Ct level by almost 5 Ct. We believe this is evidence that application of 0.5% MCFA blend or 0.3% C8 may render PEDV noninfectious. However, it is important to remember that PCR and bioassays are infection models but have not been demonstrated in large-scale commercial conditions.

In a series of previous experiments using similar inoculation, processing, and molecular diagnostic techniques, the standard deviation (standard error of the mean × square root of the number of observations per treatment) ranged from 0.47 to 1.56 Ct (Gebhardt et al., 2018, 2020 Cochrane et al., 2020). In the current series of experiments, the calculated standard deviations were similar to previous reports (0.80, 0.61, and 1.25 in exp. 1, 2, and 3, respectively). Using these measures of variability, the magnitude of difference in Ct value between two groups necessary for the desired level of statistical significance can be calculated using a two-sided sample size calculation as described by Kadam and Bhalerao (2010). If assuming a desired power of 80% (1-β) and $\alpha = 0.05$ with a baseline PC Ct value assumed to be 27, the magnitude of difference in Ct values between a treatment group and control necessary for statistical significance would range from 1.4 to 2.9 (27.0 vs. 28.4 if the smallest standard deviation in the current series of experiments of 0.61 is used in the calculation; 27.0 vs. 29.9 if the largest standard deviation in the current series of experiments of 1.25 is used in the calculation). In the current series of experiments, differences ranging from 1.4 to 2.9 Ct between treatments or greater were observed, and the body of literature suggests that differences of this magnitude or greater can commonly be seen with mitigation strategies as currently evaluated. Thus, the current model is a scientifically valid approach for evaluating differences in the detection of PEDV genetic material using three replicates per treatment combination.

These experiments demonstrate that MCFA are effective at reducing detectable PEDV via qRT-PCR both before and after virus inoculation. This is an important finding for the swine industry when considering that feed could be contaminated either before chemical application due to ingredient contamination or after manufacturing due to mill or equipment contamination. Lastly, we observed that a 1:1:1 blend of hexanoic, octanoic, and decanoic acid remains a promising option to reduce PEDV in feed, preventing infection at a 0.5% application level. Individually, C6:0 and C8:0 seem to be delivering a majority of this antiviral activity. The formaldehyde-based product, 0.5% C6:C8:C10 blend in a 1:1:1 ratio, and 0.3% C8:0 prevented infection in a bioassay. Further research should continue to validate the lower inclusion levels of MCFA to prevent viral transmission in swine feed in order to increase the economic feasibility of their application.

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Conflict of interest statement

B.B., T.K., and B.D.R. are employees of PMI, the company who partially funded this research. All other authors declare no conflict of interest.

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