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Effects of Medium Chain Fatty Acid Application in Swine Feed on Porcine Epidemic Diarrhea Virus

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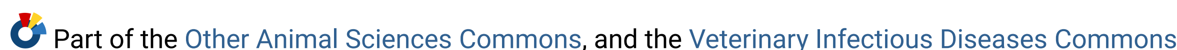

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Effects of Medium Chain Fatty Acid Application in Swine Feed on Porcine Epidemic Diarrhea Virus

Abstract

Medium chain fatty acid (MCFA) application has been identified as a promising strategy to decrease viral pathogen transmission in swine feed. Four experiments were conducted to: 1) determine if MCFAs are effective when applied to feed both prior to and after porcine epidemic diarrhea virus (PEDV) inoculation measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR), 2) evaluate the effects of varying amounts and combinations of MCFA measured by qRT-PCR, and 3) evaluate selected MCFA treatments in a bioassay. In Exp. 1, treatments were arranged in a 2 × 2 + 1 factorial with the main effects of chemical treatment (0.3% Sal CURB [Kemin Industries, Des Moines, IA] or 1% MCFA blend of 1:1:1 C6:C8:C10 [PMI, Arden Hills, MN]) and timing of chemical treatment (pre or post-inoculation with PEDV), plus a positive control (feed inoculated with PEDV and no chemical treatment). Feed was treated with the respective treatment either before or after inoculation at which point it remained at ambient temperature for 24 h and then was analyzed via qRT-PCR. The analyzed values represent cycle threshold (Ct), for which a lower number indicates greater detection of viral nucleic acid. Results demonstrated that all combinations of chemical treatment and timing increased Ct compared to the positive control ($P < 0.05$). Additionally, treatment of feed pre-PEDV inoculation resulted in increased Ct value compared to post- inoculation treatment ($P = 0.009$) and Sal CURB increased Ct in comparison with 1% MCFA ($P < 0.0001$). In Exp. 2, the chemical treatments were applied pre-inoculation and consisted of:1) positive control, 2) 0.3% Sal CURB, 3) 0.125% C6, 4) 0.25% C6, 5) 0.33% C6,6) 0.125% C8, 7) 0.25% C8, 8) 0.33% C8, 9) 0.125% C10, 10) 0.25% C10, 11) 0.33% C10, 12) 0.125% C5, 13) 0.25% C5, 14) 0.33% C5, and 15) 0.66% C5, which were analyzed via qRT-PCR. Treatment of feed with 0.33% C8 resulted in increased ($P < 0.05$) Ct values compared to all other levels of MCFA and the positive control feed. Further, Sal CURB, 0.25% C6, 0.33% C6, all levels of C8, 0.25% C10, 0.33% C10, or 0.66% C5 all had increased Ct values compared to positive control feed ($P < 0.05$). Increasing amounts of each individual MCFA resulted in increased Ct ($P < 0.045$). In Exp. 3, the chemical treatments were applied pre-inoculation and consisted of:

1) positive control; 2) 0.3% Sal CURB; 3) 0.25% MCFA blend; 4) 0.375% MCFA blend; 5) 0.500% MCFA blend; 6) 0.750% MCFA blend; 7) 1.0% MCFA blend; 8) 0.125% C6 + 0.125% C8; 9) 0.25% C6 + 0.25% C8; 10) 0.33% C6 + 0.33% C8; 11) 0.125% C6 + 0.125% C10; 12) 0.25% C6 + 0.25% C10; 13) 0.33% C6 + 0.33% C10; 14) 0.125% C8 + 0.125% C10; 15) 0.25% C8 + 0.25% C10; and 16) 0.33% C8 + 0.33% C10, which were analyzed via qRT-PCR. Treating feed with Sal CURB, 0.500% blend, 0.750% blend, 1.0% blend, all levels of the C6 + C8, 0.25% C6 + 0.25% C10, 0.33% C6 + 0.33% C10, 0.25% C8 + 0.25% C10, or 0.33% C8 + 0.33% C10 resulted in increased Ct compared to the positive control ($P < 0.05$). Lastly, in Exp. 4, feed was treated pre-inoculation with either 1) no treatment (positive control); 2) 0.3% Sal CURB; 3) 0.5% MCFA blend; or 4) 0.3% C8 and samples were analyzed via qRT-PCR and bioassay. Adding either 0.5% MCFA blend or 0.3% C8 resulted in increased Ct compared to the positive control. Further, only the positive control resulted in a positive in vivo bioassay. This set of experiments demonstrates that MCFA and Sal CURB are effective at decreasing detection of PEDV in feed both prior to and post-inoculation. Additionally, inclusion of lower levels of MCFA than previously evaluated may provide protection against PEDV transmission through feed.

Keywords

medium chain fatty acids, PEDV, feed, swine

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Cover Page Footnote
Appreciation is expressed to PMI (Arden Hills, MN) for financial support of these projects.

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Effects of Medium Chain Fatty Acid Application in Swine Feed on Porcine Epidemic Diarrhea Virus[1]

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Summary

Medium chain fatty acid (MCFA) application has been identified as a promising strategy to decrease viral pathogen transmission in swine feed. Four experiments were conducted to: 1) determine if MCFAs are effective when applied to feed both prior to and after porcine epidemic diarrhea virus (PEDV) inoculation measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR), 2) evaluate the effects of varying amounts and combinations of MCFA measured by qRT-PCR, and 3) evaluate selected MCFA treatments in a bioassay.

In Exp. 1, treatments were arranged in a 2 × 2 + 1 factorial with the main effects of chemical treatment (0.3% Sal CURB [Kemin Industries, Des Moines, IA] or 1% MCFA blend of 1:1:1 C6:C8:C10 [PMI, Arden Hills, MN]) and timing of chemical treatment (pre or post-inoculation with PEDV), plus a positive control (feed inoculated with PEDV and no chemical treatment). Feed was treated with the respective treatment either before or after inoculation at which point it remained at ambient temperature for 24 h and then was analyzed via qRT-PCR. The analyzed values represent cycle threshold (Ct), for which a lower number indicates greater detection of viral nucleic acid. Results demonstrated that all combinations of chemical treatment and timing increased Ct compared to the positive control ($P < 0.05$). Additionally, treatment of feed pre-PEDV inoculation resulted in increased Ct value compared to post-inoculation treatment ($P = 0.009$) and Sal CURB increased Ct in comparison with 1% MCFA ($P < 0.0001$).

[1] Appreciation is expressed to PMI (Arden Hills, MN) for financial support of these projects.

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In Exp. 2, the chemical treatments were applied pre-inoculation and consisted of: 1) positive control, 2) 0.3% Sal CURB, 3) 0.125% C6, 4) 0.25% C6, 5) 0.33% C6, 6) 0.125% C8, 7) 0.25% C8, 8) 0.33% C8, 9) 0.125% C10, 10) 0.25% C10, 11) 0.33% C10, 12) 0.125% C5, 13) 0.25% C5, 14) 0.33% C5, and 15) 0.66% C5, which were analyzed via qRT-PCR. Treatment of feed with 0.33% C8 resulted in increased ($P < 0.05$) Ct values compared to all other levels of MCFA and the positive control feed. Further, Sal CURB, 0.25% C6, 0.33% C6, all levels of C8, 0.25% C10, 0.33% C10, or 0.66% C5 all had increased Ct values compared to positive control feed ($P < 0.05$). Increasing amounts of each individual MCFA resulted in increased Ct ($P < 0.045$).

In Exp. 3, the chemical treatments were applied pre-inoculation and consisted of: 1) positive control; 2) 0.3% Sal CURB; 3) 0.25% MCFA blend; 4) 0.375% MCFA blend; 5) 0.500% MCFA blend; 6) 0.750% MCFA blend; 7) 1.0% MCFA blend; 8) 0.125% C6 + 0.125% C8; 9) 0.25% C6 + 0.25% C8; 10) 0.33% C6 + 0.33% C8; 11) 0.125% C6 + 0.125% C10; 12) 0.25% C6 + 0.25% C10; 13) 0.33% C6 + 0.33% C10; 14) 0.125% C8 + 0.125% C10; 15) 0.25% C8 + 0.25% C10; and 16) 0.33% C8 + 0.33% C10, which were analyzed via qRT-PCR. Treating feed with Sal CURB, 0.500% blend, 0.750% blend, 1.0% blend, all levels of the C6 + C8, 0.25% C6 + 0.25% C10, 0.33% C6 + 0.33% C10, 0.25% C8 + 0.25% C10, or 0.33% C8 + 0.33% C10 resulted in increased Ct compared to the positive control ($P < 0.05$).

Lastly, in Exp. 4, feed was treated pre-inoculation with either 1) no treatment (positive control); 2) 0.3% Sal CURB; 3) 0.5% MCFA blend; or 4) 0.3% C8 and samples were analyzed via qRT-PCR and bioassay. Adding either 0.5% MCFA blend or 0.3% C8 resulted in increased Ct compared to the positive control. Further, only the positive control resulted in a positive in vivo bioassay.

This set of experiments demonstrates that MCFA and Sal CURB are effective at decreasing detection of PEDV in feed both prior to and post-inoculation. Additionally, inclusion of lower levels of MCFA than previously evaluated may provide protection against PEDV transmission through feed.

Introduction

Recently published literature regarding viral transmission in swine feed has generated increased interest in determining the effects of chemical mitigants and feed additives on virus quantification and infectivity. 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. Cochrane et al.[6] demonstrated the efficacy of MCFA as an effective strategy to decrease detectable viral nucleic acid and virus infectivity in complete swine feed. Adding 1% MCFA blend containing caproic (C6), caprylic (C8), and capric (C10) acids in a 1:1:1 ratio significantly reduced PEDV RNA levels in swine feed when

[6] Cochrane, R. A.; Dritz, S. S.; Woodworth, J. C.; Huss, A. R.; Stark, C. R.; Saensukjaroenphon, M.; DeRouchey, J. M.; Tokach, M. D.; Goodband, R. D.; Bai, J.; Chen, Qi; Zhang, Jianqiang; Gauger, Phillip Charles; Derscheid, Rachel J.; Main, Rodger G.; and Jones, C. K. (2016) "Assessing the Effects of Medium Chain Fatty Acids and Fat Sources on Porcine Epidemic Diarrhea Virus Viral RNA Stability and Infectivity," Kansas Agricultural Experiment Station Research Reports: Vol. 2: Iss. 8. https://doi.org/10.4148/2378-5977.1278.

the MCFA blend was applied prior to inoculation.[5] Gebhardt et al.[7] also observed a decrease in detectable virus when 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 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 these 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 bioassay.

Procedures

Chemical Treatments

Experiment 1

Chemical treatments included in Exp. 1 were Sal CURB (0.3%, Kemin Industries, Des Moines, IA) and 1% MCFA blend (1:1:1 ratio of C6:C8:C10 (PMI, Arden Hills, MN)) applied either pre- or post-inoculation with PEDV. Pre-inoculation chemical treatments were applied 24 h before virus inoculation. Post-inoculation chemical treatments were applied within 1 h after virus inoculation then shaken to ensure even dispersion, and then stored overnight. There were six replications (250 mL bottles) per treatment.

Experiment 2

Chemical treatments (administered prior to viral inoculation) included in Exp. 2 were as follows:

1. Non-treated, PEDV inoculated control (positive control)
2. Sal CURB (0.3%)
3. 0.125% C6
4. 0.25% C6
5. 0.33% C6
6. 0.125% C8
7. 0.25% C8
8. 0.33% C8
9. 0.125% C10
10. 0.25% C10
11. 0.33% C10
12. 0.125% C5
13. 0.25% C5
14. 0.33% C5
15. 0.66% C5

There were four replications per treatment.

[7] Gebhardt, J. T.; Woodworth, J. C.; Tokach, M. D.; DeRouchey, J. M.; Goodband, R. D.; Jones, C. K.; and Dritz, S. S. (2017) "Quantifying Medium Chain Fatty Acid Mitigation Activity Over Time against Porcine Epidemic Diarrhea Virus in Nursery Pig Diets," Kansas Agricultural Experiment Station Research Reports: Vol. 3: Iss. 7. https://doi.org/10.4148/2378-5977.7464.

Experiment 3

Chemical treatments (administered prior to viral inoculation) included in Exp. 3 were as follows:

1. Positive control
2. Sal CURB (0.3%)
3. 0.25% MCFA blend (1:1:1 ratio of C6:C8:C10)
4. 0.375% MCFA blend (1:1:1 ratio of C6:C8:C10)
5. 0.500% MCFA blend (1:1:1 ratio of C6:C8:C10)
6. 0.750% MCFA blend (1:1:1 ratio of C6:C8:C10)
7. 1.0% MCFA blend (1:1:1 ratio of C6:C8:C10)
8. 0.125% C6 + 0.125% C8
9. 0.25% C6 + 0.25% C8
10. 0.33% C6 + 0.33% C8
11. 0.125% C6 + 0.125% C10
12. 0.25% C6 + 0.25% C10
13. 0.33% C6 + 0.33% C10
14. 0.125% C8 + 0.125% C10
15. 0.25% C8 + 0.25% C10
16. 0.33% C8 + 0.33% C10

With the exception of the MCFA blend treatments, MCFA in combination were added individually. There were four replications per treatment.

Experiment 4

Treatments (administered prior to viral inoculation) for the bioassay included the following:

1. Positive control
2. Sal CURB (0.3%)
3. 0.5% MCFA blend (1:1:1 ratio of C6:C8:C10)
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 Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. A new batch of feed was manufactured for each experiment. Chemical treatments were applied to 100 g of feed, which was then mixed using a mason jar feed mixer (Central Machine Shop, Purdue University, West Lafayette, IN) using 10 hex nuts to ensure agitation with a dry mix time of 15 minutes. 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.

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.[8] 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 (Exp. 1, 2, and 3) and the Iowa State University Veterinary Diagnostic Laboratory (Exp. 4). All treatments were inoculated using an appropriately sized pipette 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 pre-treatment bottles received viral inoculation 24 h after chemical treatment, whereas the post-inoculation chemical treatments were applied within 1 hr of viral inoculation. Bottles were then shaken for 15 seconds to further distribute virus throughout feed.

Real Time PCR Analysis

All bottles were then kept at ambient temperature and at 24 h post inoculation, 100 mL of phosphate buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY) was placed in each bottle containing 22.5 g of inoculated feed. Samples were swirled to ensure even mixing and stored at 4°C for 24 hours at which point supernatant was collected and stored at -80°C until qRT-PCR or bioassay was performed.

Quantitative real time reverse transcription polymerase chain reaction (qRT-PCR) procedures were conducted as previously described from Gebhardt et al.[6] Fifty microliters (µL) 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 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 greater nucleic acid detection, but not necessarily infectivity.

Bioassay

Bioassay procedures in Exp. 4 were replicated from Cochrane et al.[5] The Iowa State University Institutional Animal Care and Use Committee reviewed and approved the pig bioassay protocol. Fifteen, crossbred commercial pigs (10 d of age) of mixed sex were obtained from a sow herd with no prior exposure to PEDV. Pigs were confirmed to be negative for PEDV, porcine delta coronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV) by a PCR-based test of fecal swabs upon arrival. Pigs were also confirmed to be PEDV antibody negative by an PEDV S1 fluorescent multiplex immunoassay (FMIA) on serum samples. All assays were conducted at the Iowa State University Veterinary Diagnostic Laboratory. Pigs were allowed 2 d of acclimation upon arrival before the bioassay began. A total of 5 rooms (3 pigs/room; 15 pigs total) were assigned to treatment groups with 1 negative control room and 4 challenge rooms. During bioassays, rectal swabs were collected on d -2, 0, 3, 5, and 7 days post-inocula-

[8] Chen et al., 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: 234-243.

tion (dpi) from all pigs and tested for PEDV RNA via qRT-PCR. Following humane euthanasia at 7 dpi, cecal contents were collected.

Statistical Analysis

For Exp. 1, the main effects of chemical mitigant and timing of chemical application were also evaluated. In Exp. 2 and 3, overall treatment effect was evaluated in addition to linear and quadratic responses with increasing doses of individual or combination MCFA.

All data were analyzed for the fixed effect of chemical treatment using PROC GLIMMIX in SAS (v. 9.4, SAS Institute, Inc. Cary, NC). Results were considered significant at $P < 0.05$ and marginally significant at $P > 0.05$ and $P < 0.10$.

Results and Discussion

Experiment 1

Interactive effects of timing of chemical treatment and type of chemical treatment are presented in Table 1. Pre-inoculation treatment with Sal CURB resulted in an increased ($P < 0.05$) Ct value compared to pre- and post-inoculation MCFA application, and the positive control feed, but there was no evidence for difference when compared to post-inoculation Sal CURB application. There was no evidence pre-inoculation MCFA treatment resulted in a different Ct value compared to post-inoculation Sal CURB treatment. Pre-inoculation MCFA increased ($P < 0.05$) Ct value compared with post-inoculation MCFA application and the positive control feed. Lastly, the post-inoculation MCFA-treated feed had increased ($P < 0.05$) Ct value compared with positive control feed. When evaluating main effects of timing of treatments, treating feed with chemical prior to PEDV inoculation resulted in an increased ($P = 0.009$; Table 2) Ct value, or less detectable viral RNA, than feed treated with chemical after PEDV inoculation (Table 2). Also, regardless of time of application, Sal CURB resulted in increased ($P < 0.0001$) Ct value compared with MCFA-treated feed (Table 3).

Experiment 2

There was a significant effect ($P < 0.001$; Table 4) of MCFA level and chemical treatment (applied pre-inoculation) on the detectable PEDV RNA (Table 4). Feed treatment with 0.33% C8 resulted in increased ($P < 0.05$) Ct values compared to all other levels of MCFA and the positive control feed. Alternatively, Sal CURB, 0.25% C6, 0.33% C6, all levels of C8, 0.25% C10, 0.33% C10, and 0.66% C5 all had increased Ct values compared to positive control feed ($P < 0.05$). Further, increasing C6 addition from 0.125 to 0.33% resulted in increased (linear, $P = 0.003$) Ct values. Increasing C8 and C10 addition resulted in a quadratic increase in Ct ($P < 0.045$). Lastly, increasing C5 from 0.125 and 0.66% also resulted in linear increases in Ct values ($P = 0.021$).

Experiment 3

When evaluating MCFA in combination and varying concentrations applied pre-inoculation, there was a significant effect of treatment ($P < 0.0001$; Table 5). Treatments that had significantly increased ($P < 0.05$) Ct values compared to the positive control feed included Sal CURB; 0.50% blend; 0.75% blend; 1.0% blend; all levels of the C6 + C8; 0.25% C6 + 0.25% C10; 0.33% C6 + 0.33% C10; 0.25% C8 + 0.25%

C10; and 0.33% C8 + 0.33% C10. Increasing MCFA blend resulted in increasing Ct (linear, $P = 0.001$). Increasing combination of C6 + C8 from 0.25 to 0.66% resulted in a marginally significant increase in Ct (linear, $P = 0.052$). There was no evidence that increasing the combination of C6 + C10 affected Ct (linear, $P = 0.115$). Lastly, increasing C8 + C10 resulted in a marginally significant quadratic increase in Ct ($P = 0.097$).

Experiment 4

The qRT-PCR results demonstrated a significant effect of pre-inoculation chemical treatment on feed ($P < 0.0001$; Table 6), with 0.5% MCFA blend and 0.3% C8 having increased ($P < 0.05$) Ct compared to the positive control and Sal CURB treatments. For the bioassay, as expected, pigs inoculated with supernatant from negative control did not have positive PEDV bioassay results. Pigs inoculated with positive control feed resulted in PEDV infection. For all other treatments there was no evidence of PEDV infection detected for fecal swabs and cecal contents.

Conclusion

These experiments demonstrated that MCFA and Sal CURB are effective at reducing detectable PEDV quantified via qRT-PCR both before and after virus inoculation. This is an important finding for the swine industry when considering that feed could be infected 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 caproic, caprylic, and capric acid remains a promising option to reduce PEDV in feed. Depending on concentration and composition, MCFAs had similar impacts on increasing Ct value compared to Sal CURB. However, C8 appears to be driving the majority of this antiviral activity.

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Table 1. Effect of MCFA or Sal CURB and timing of treatment in relation to PEDV inoculation on PEDV detection using qRT-PCR (Exp. 1)[1,2]

		Pre-inoculation[3]		Post-inoculation[3]			
Item	Positive control[4]	MCFA[5]	Sal CURB[6]	MCFA	Sal CURB	SEM	*P* <
qRT-PCR, Ct[7]	26.5[d]	30.6[b]	32.4[a]	28.8[c]	31.5[a,b]	0.457	<0.0001

[1]A total of 30 samples (6 samples per treatment) were used.
[2]An initial tissue culture (2.5 mL diluted PEDV inoculum, 10^5 $TCID_{50}$/mL) was added to 22.5 grams of swine diet treated with either an MCFA blend or Sal CURB.
[3]Pre-inoculation indicates that the chemical treatments were applied 24 h before inoculation with PEDV. Post-inoculation indicates that chemical treatments were applied within 1 h after inoculation with PEDV.
[4]Positive control = non-chemically treated feed inoculated with PEDV.
[5]MCFA treatment consisted of a 1:1:1 blend of C6:C8:C10 (caproic, caprylic, and capric acids, respectively) applied to swine feed at an addition of 1%.
[6]Sal CURB (Kemin Industries, Des Moines, IA) was applied to swine feed at 0.3% of the diet.
[7]Cycle threshold (Ct) required to detect viral nucleic acid. A high Ct value indicates less viral nucleic acid present.
[abcd]Means with differing superscripts differ $P < 0.05$.
MCFA = medium chain fatty acid. PEDV = porcine epidemic diarrhea virus. qRT-PCR = quantitative real time reverse transcription polymerase chain reaction.

Table 2. Main effect of timing of chemical application (pre- or post-inoculation) on PEDV detection using qRT-PCR (Exp. 1)[1,2]

Item	Pre-inoculation[3]	Post-inoculation[3]	SEM	*P* =
qRT-PCR, Ct[4]	31.5	30.2	0.323	0.009

[1]A total of 24 samples (12 per inoculation timing method) were used.
[2]An initial tissue culture (2.5 mL diluted PEDV inoculum, 10^5 $TCID_{50}$/mL) was added to 22.5 grams of swine diet.
[3]Pre-inoculation indicates that the chemical treatments were applied 24 h prior to inoculation with PEDV. Post-inoculation indicates that chemical treatments were applied within 1 h after inoculation with PEDV.
[4]Cycle threshold (Ct) required to detect viral nucleic acid. A high Ct value indicates less viral nucleic acid present.
MCFA = medium chain fatty acid. PEDV = porcine epidemic diarrhea virus. qRT-PCR = quantitative real time reverse transcription polymerase chain reaction.

Table 3. Main effect of chemical mitigant used to treat swine feed on PEDV detection using qRT-PCR (Exp. 1)[1,2]

Item	Sal CURB[3]	MCFA[4]	SEM	*P* =
qRT-PCR, Ct[5]	31.9	29.7	0.32	<0.0001

[1]A total of 24 samples (12 per chemical treatment) were used.
[2]An initial tissue culture (2.5 mL diluted PEDV inoculum, 10^5 $TCID_{50}$/mL) was added to 22.5 grams of swine diet and treated with either a MCFA blend or Sal CURB.
[3]Sal CURB (Kemin Industries, Des Moines, IA) was applied to feed at 0.3% inclusion rate.
[4]MCFA treatment consisted of a 1:1:1 blend of C6:C8:C10 (caproic, caprylic, and capric acids, respectively) applied to the feed at a 1% inclusion rate.
[5]Cycle threshold (Ct) required to detect viral genetic material. A high Ct value indicates less genetic material present.
MCFA = medium chain fatty acid. PEDV = porcine epidemic diarrhea virus. qRT-PCR = quantitative real time reverse transcription polymerase chain reaction.

Table 4. Effect of chemical mitigant used to treat swine feed on PEDV detection using qRT-PCR (Exp. 2)[1,2,3]

Item	qRT-PCR, Ct[4]	SEM	$P =$
Positive control[5]	27.2[g]	0.346	<0.0001
Sal CURB[6]	29.3[b]		
C6[7]			
0.125%	27.8[defg]		
0.25%	28.9[bc]		
0.33%	29.4[b]		
C8[8]			
0.125%	28.8[bcd]		
0.25%	29.0[bc]		
0.33%	31.3[a]		
C10[9]			
0.125%	27.7[efg]		
0.25%	28.4[bced]		
0.33%	27.4[fg]		
C5[10]			
0.125%	27.1[g]		
0.25%	27.2[fg]		
0.33%	27.3[fg]		
0.66%	28.3[cdef]		

[1]A total of 60 (4 per treatment) samples were used. Feed was confirmed to be negative for porcine epidemic diarrhea virus (PEDV) via quantitative real time reverse transcription polymerase chain reaction (qRT-PCR).
[2]Feed was confirmed to be negative Ct > 36.
[3]An initial tissue culture (2.5 mL diluted PEDV inoculum, 10^5 $TCID_{50}$/mL) was added to 22.5 grams of swine diet previously treated with varying levels of C6, C8, C10, or no feed additive treatment.
[4]Cycle threshold (Ct) required to detect viral nucleic acid. A high Ct value indicates less viral nucleic acid present.
[5]Positive control = non-chemically treated feed inoculated with PEDV.
[6]Sal CURB (Kemin Industries, Des Moines, IA) was applied to feed at 0.3% inclusion rate.
[7]C6, linear, $P = 0.003$; quadratic, $P = 0.430$.
[8]C8, linear, $P < 0.0001$; quadratic, $P = 0.015$.
[9]C10, linear, $P = 0.630$; quadratic, $P = 0.045$.
[10]C5, linear, $P = 0.021$; quadratic, $P = 0.209$.
[abcdefg]Means with differing superscripts differ ($P < 0.05$).

Table 5. Effect of chemical mitigant used to treat swine feed on PEDV detection using qRT-PCR (Exp. 3)[1,2,3]

Item	qRT-PCR, Ct[4]	SEM	*P* =
Positive control[5]	27.8[f]	0.718	<0.0001
Sal CURB[6]	32.7[ab]		
MCFA Blend, %[7]			
0.250	29.7[def]		
0.375	29.4[def]		
0.500	32.3[abc]		
0.750	31.8[abc]		
1.000	33.2[a]		
C6 + C8, %[8,9]			
0.125	30.7[bcde]		
0.25	31.4[abcd]		
0.33	32.7[ab]		
C6 + C10, %[10]			
0.125	29.3[ef]		
0.25	30.4[cde]		
0.33	30.9[bcde]		
C8 + C10, %[11]			
0.125	29.4[ef]		
0.25	31.3[abcde]		
0.33	30.3[cde]		

[1]A total of 64 samples (4 per treatment) were used. Feed was confirmed to be negative for porcine epidemic diarrhea virus (PEDV) via quantitative real time reverse transcription polymerase chain reaction (qRT-PCR).
[2]Feed was confirmed to be negative Ct > 36.
[3]An initial tissue culture (2.5 mL diluted PEDV inoculum, 10^5 $TCID_{50}$/mL) was added to 22.5 grams of swine diet previously treated with varying levels of C6, C8, or C10, combinations of a blend of C6:C8:C10, or no feed additive treatment.
[4]Cycle threshold (Ct) required to detect viral nucleic acid. A higher Ct value indicates less viral nucleic acid present.
[5]Positive control = non-chemically treated feed inoculated with PEDV.
[6]Sal CURB (Kemin Industries, Des Moines, IA) was applied to feed at 0.3% inclusion rate.
[7]Medium chain fatty acid (MCFA) treatment consisted of a 1:1:1 blend of C6:C8:C10 (caproic, caprylic, and capric acids, respectively) applied to the feed at a 1% inclusion rate. MCFA blend, linear, $P = 0.001$; quadratic, $P = 0.999$.
[8]The percent indicates the amount of each MCFA added to the feed.
[9]C6 + C8, linear, $P = 0.052$; quadratic, $P = 0.743$.
[10]C6 + C10, linear, $P = 0.115$; quadratic, $P = 0.773$.
[11]C8 + C10, linear, $P = 0.351$; quadratic, $P = 0.097$.
[abcdef]Means with differing superscripts differ ($P < 0.05$).

Table 6. Effect of chemical mitigant used to treat swine feed on PEDV detection using qRT-PCR and pig bioassay (Exp. 4)[1]

		Fecal swabs					Cecal content,
Item	Feed Ct[2,3]	-2 dpi[4]	0 dpi	3 dpi	5dpi	7 dpi	7 dpi[5]
Negative control	> 36	---[6]	---	---	---	---	> 36
Positive control[7]	28.0[b]	---	---	+--	++-	+--	25.4[8]
Sal CURB[9]	29.2[b]	---	---	---	---	---	> 36
0.5% MCFA Blend[10]	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. Three feed samples per treatment were collected and diluted in phosphate buffered saline. The supernatant from each sample was then collected for pig bioassay. The supernatant was administered one time via oral gavage on d 0 to each of three pigs per treatment (10 mL per pig). Thus, the cecum contents are represented by a mean of 3 pigs per treatment. Pigs were inoculated at d 12 of age.
[2]A cycle threshold (Ct) > 36 was considered negative for presence of PEDV RNA. Feed Ct analysis was carried out at Kansas State University College of Veterinary Medicine Virology Laboratory .
[3]All treatments with the exception of the negative control were analyzed for the fixed effect of treatment. $P < 0.0001$.
[4]dpi = day post-inoculation.
[5]A Ct greater than 36 was considered negative for PEDV RNA. PCR and bioassay were conducted at the Iowa State University Veterinary Diagnostic Laboratory.
[6]A (-) indicates a negative fecal swab from one pig whereas a (+) indicates a PEDV positive fecal swab.
[7]Positive control = non-chemically treated feed inoculated with PEDV.
[8]One pig had cecal contents that resulted in 25.4 Ct, while the other two pigs had negative cecal contents.
[9]Sal CURB (Kemin Industries, Des Moines, IA) was applied to feed at 0.3%.
[10]Medium chain fatty acid (MCFA) blend consisted of a 1:1:1 blend of C6:C8:C10 (caproic, caprylic, and capric acids, respectively) applied to the feed at a 0.5%.
[ab]Means with differing superscripts within column differ ($P < 0.05$).
PEDV = porcine epidemic diarrhea virus. qRT-PCR = quantitative real time reverse transcription polymerase chain reaction.