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Assessing the Effects of Medium Chain Fatty Acids and Fat Sources on Porcine Epidemic Diarrhea Virus Viral RNA Stability and Infectivity

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Abstract

Research has confirmed that chemical treatments, such as medium chain fatty acids (MCFA) and commercial formaldehyde, can be effective to reduce the risk of porcine epidemic diarrhea virus (PEDV) cross-contamination in feed. However, the efficacy of individual MCFA levels are unknown. The objective of this study is to compare the efficacy of commercially-available sources of MCFA and other fat sources versus a synthetic custom blend of MCFA to minimize the risk of PEDV cross-contamination as measured by qRT-PCR and bioassay. Treatments were arranged in a 17 × 4 plus 1 factorial with 17 chemical treatments: 1) Positive control with PEDV and no chemical treatment, 2) 0.3% Sal CURB, 3) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (aerosolized), 4) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (non-aerosolized), 5) 0.66% caproic acid, 6) 0.66% caprylic acid, 7) 0.66% capric acid, 8) 0.66% lauric acid, 9) 1% capric and lauric acid mixture (1:1 ratio), 10) FRA C12, 11) 1% choice white grease, 12) 1% soy oil, 13) 1% canola oil, 14) 2% palm kernel oil, 15) 1% palm kernel oil, 16) 2% coconut oil, and 17) 1% coconut oil; 4 analysis days of 0, 1, 3, and 7 post inoculation; and 1 treatment of PEDV negative, untreated feed. Matrices were first chemically treated, then inoculated with PEDV, and stored at room temperature until being analyzed by qRT-PCR. The analyzed values represent threshold cycle (CT), at which a higher CT value represents less detectable RNA. All main effects and interactions were significant ($P < 0.002$). The interaction of treatment × day indicated that over time the MCFA treatments, either as a mixture or as individual fatty acids, and Sal CURB had the greatest effect of reducing detectable PEDV RNA, which follows the same trend as the main effect of treatment and the bioassay results. Feed treated with individual synthetic MCFA, MCFA mixture, or Sal CURB had fewer ($P < 0.05$) detectable viral particles than all other treatments. Day also had a significant impact on quantification of viral RNA, and CT increased from 29.5 to 34.6 CT from day 0 to 7, respectively. In summary, time, Sal CURB, 1% MCFA, 0.66% caproic, 0.66% caprylic, and 0.66% capric acids enhance the RNA degradation of PEDV in swine feed. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially-available formaldehyde product in the complete swine diet at 1% inclusion and as individual fatty acids.

Keywords
PEDV, medium chain fatty acids, fat source, swine

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Assessing the Effects of Medium Chain Fatty Acids and Fat Sources on Porcine Epidemic Diarrhea Virus Viral RNA Stability and Infectivity[1]

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Summary

Research has confirmed that chemical treatments, such as medium chain fatty acids (MCFA) and commercial formaldehyde, can be effective to reduce the risk of porcine epidemic diarrhea virus (PEDV) cross-contamination in feed. However, the efficacy of individual MCFA levels are unknown. The objective of this study is to compare the efficacy of commercially-available sources of MCFA and other fat sources versus a synthetic custom blend of MCFA to minimize the risk of PEDV cross-contamination as measured by qRT-PCR and bioassay. Treatments were arranged in a 17 × 4 plus 1 factorial with 17 chemical treatments: 1) Positive control with PEDV and no chemical treatment, 2) 0.3% Sal CURB, 3) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (aerosolized), 4) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (non-aerosolized), 5) 0.66% caproic acid, 6) 0.66% caprylic acid, 7) 0.66% capric acid, 8) 0.66% lauric acid, 9) 1% capric and lauric acid mixture (1:1 ratio), 10) FRA C12, 11) 1% choice white grease, 12) 1% soy oil, 13) 1% canola oil, 14) 2% palm kernel oil, 15) 1% palm kernel oil, 16) 2% coconut oil, and 17) 1% coconut oil; 4 analysis days of 0, 1, 3, and 7 post inoculation; and 1 treatment of PEDV negative, untreated feed. Matrices were first chemically treated, then inoculated with PEDV, and stored at room temperature until being analyzed by qRT-PCR. The analyzed values represent threshold cycle (CT), at which a higher CT value represents less detectable RNA. All main effects and interactions were significant ($P < 0.002$). The interaction of treatment × day indicated that over time the MCFA treatments, either as a mixture or as individual fatty acids, and Sal CURB had the greatest effect of reducing detectable PEDV RNA, which follows the same trend as the main effect

of treatment and the bioassay results. Feed treated with individual synthetic MCFA, MCFA mixture, or Sal CURB had fewer ($P < 0.05$) detectable viral particles than all other treatments. Day also had a significant impact on quantification of viral RNA, and CT increased from 29.5 to 34.6 CT from day 0 to 7, respectively. In summary, time, Sal CURB, 1% MCFA, 0.66% caproic, 0.66% caprylic, and 0.66% capric acids enhance the RNA degradation of PEDV in swine feed. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially-available formaldehyde product in the complete swine diet at 1% inclusion and as individual fatty acids.

Key words: PEDV, medium chain fatty acids, fat source, swine

Introduction

Porcine Epidemic Diarrhea Virus (PEDV) is an enveloped single-stranded positive-sense RNA virus that was first identified in the United States in May 2013. Epidemiological and controlled experiments have shown that complete feed or feed components can be one of many possible vectors of transmission of PEDV.[5] Previous research has shown that a 2% and 1% mixture of caproic, caprylic, and capric acids can reduce the risk of PEDV in a complete swine diet.[6] However, it has not been established if the response observed from the medium chain fatty acid (MCFA) treatment is due to unique characteristics of those particular fatty acids, or if the response is due to increasing the total quantity of fat in the diet. Furthermore, the synthetic blend of MCFA previously tested is not commercially available and may be cost-prohibitive to employ, so further evaluation of the mode-of-action of MCFA and potential replacement with commercially-available sources is warranted. Therefore, the objective of this study is to compare the efficacy of commercially available sources of MCFA and other fat sources versus a synthetic custom blend of MCFA to minimize the risk of PEDV cross-contamination as measured by qRT-PCR and bioassay.

Procedures

In order to evaluate the use of chemical treatments and fat sources on PEDV survival, a corn-soybean meal-based swine diet was used and manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. The diet was first chemically treated before inoculation with PEDV in order to mimic post-processing contamination.

Chemical Treatment

Eighteen chemical treatments were applied to the diet and analyzed on 4 days (d 0, 1, 3, and 7 post inoculation). The 18 treatments were 1) negative control with no PEDV and no chemical; 2) positive control with PEDV and no chemical treatment; 3) 0.325% Sal CURB; Kemin Industries, Des Moines, IA; 4) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (aerosolized); 5) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (non-aerosolized); 6) 0.66% caproic acid; 7) 0.66% caprylic acid; 8) 0.66% capric acid; 9) 0.66% lauric acid; 10) 1% capric

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[5] Dee et al., 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naïve pigs following consumption via natural feeding behavior: proof of concept. BMC Veterinary Research 2014, 10:176.

[6] Cochrane 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." Journal of Animal Science 94.supplement2 (2016): 50-50.

and lauric acid mixture (1:1 ratio); 11) FRA C12; Framelco, Raamsdonksveer, Netherlands; 12) 1% choice white grease; 13) 1% soy oil; 14) 1% canola oil; 15) 2% palm kernel oil; 16) 1% palm kernel oil; 17) 2% coconut oil; and 18) 1% coconut oil.

In order to treat the feed, all treatments were added on a wt/wt basis and mixed using a lab scale paddle mixer. The Sal CURB and MCFA aerosolized treatments were mixed using an air atomizing nozzle in order to reduce the droplet size of the liquid treatments. The rest of the treatments were added directly to the mixer. All treatments were mixed for a 5-minute wet mix time to ensure a uniform and complete mix.

When the mixing was complete, a total of 22.5 g of product was collected from different locations within the mixer and added to the respective 250 mL HDPE, square, wide-mouth bottle based on day and replication. In order to reduce the potential for treatment-to-treatment cross-contamination, the mixers were cleaned with soap and water between treatments. Once the treatments were added to their respective bottle, they were allowed to sit at room temperature until inoculation.

PEDV Isolate

The U.S. PEDV prototype strain cell culture isolate USA/IN/2013/19338, passage 8 (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).[7] The stock virus titer contained 4.5×10^6 $TCID_{50}$/mL and was diluted to 10^5 $TCID_{50}$/mL.

Inoculation

The feed was inoculated using an appropriately sized pipet to allow even distribution of the virus within the feed. For the inoculation, 2.5 mL of diluted viral inoculum was placed in each 250 mL bottle containing 22.5 grams of each feed treatment, resulting in each bottle containing a PEDV concentration of 10^4 $TCID_{50}$/g of feed. The bottles were then thoroughly shaken to ensure equal dispersion of the virus within each bottle. The samples were then stored at ambient temperature until aliquoted for viral RNA expression of PEDV at 0, 1, 3, and 7 days post inoculation via qRT-PCR. For each sample day, 100 mL of chilled PBS was placed in each 250 mL bottle containing 22.5 g of inoculated feed. Samples were then shaken to thoroughly mix and chilled at 4°C overnight. Feed matrix supernatants, including two PCR samples and a bioassay sample, were then pulled and stored at -80°C until the end of the trial.

Bioassay

The Iowa State University Institutional Animal Care and Use Committee reviewed and approved the pig bioassay protocol. Based on the qRT-PCR results, 15 treatments were selected for the bioassay. The 15 treatments were 1) d 0 negative control with no PEDV and no chemical treatment; 2) d 0 positive control with PEDV and no chemical treatment; 3) d 1 positive control with PEDV and no chemical treatment; 4) d 1 0.3% Sal CURB; 5) d 1 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (non-aerosolized); 6) d 1 0.66% caproic acid; 7) d 1 0.66% caprylic acid; 8) d 1 0.66% capric acid; 9) d 1 0.66% lauric acid; 10) d 1 FRA C12; 11) d 1 1% choice white

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[7] 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.

grease; 12) d 1 1% soy oil; 13) d 1 1% canola oil; 14) d 1 1% palm kernel oil; and 15) d 1 1% coconut oil.

A total of 45 crossbred, 10 d-old pigs of mixed sex were sourced from a single commercial, crossbred farrow-to-wean herd with no prior exposure to PEDV. Additionally, all pigs were confirmed negative for PEDV, porcine delta coronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV) based on fecal swab. To further confirm PEDV-negative status, collected blood serum was analyzed for PEDV antibodies by an indirect fluorescent antibody (IFA) assay and TGEV antibodies by ELISA, both conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before the bioassay began. A total of 15 rooms (45 pigs) were assigned to treatment groups with 1 negative control room and 14 challenge rooms.

During bioassays, rectal swabs were collected on d -2, 0, 2, 4, 6, and 7 post inoculation (dpi) from all pigs and tested for PEDV RNA qRT-PCR. Following humane euthanasia at 7 dpi, small intestine, cecum, and colon samples were collected at necropsy along with an aliquot of cecal contents. One section of formalin-fixed proximal, middle, distal jejunum and ileum was collected per pig for histopathology.[7]

Statistical Analysis
Data of the main effect of treatment, day, and the interaction were analyzed as a completely randomized design using PROC GLIMMIX in SAS (SAS Institute, Inc., Cary, NC). Results for treatment criteria were considered significant at $P \leq 0.05$ and marginally significant from $P > 0.05$ to $P \leq 0.10$.

Results and Discussion
qRT-PCR Results
All main effects and interactions were significant at $P < 0.0002$. The results of the interaction between treatment and day show that the PEDV positive treatment, choice white grease (CWG), soy oil, canola oil, palm kernel oil (PKO), and coconut oil (CO) were all relatively stable over the 7 days (Table 1). However, when looking at the MCFA and Sal CURB treatments, it was observed that over time these treatments resulted in a greater reduction of the detectable genetic material ($P < 0.05$). The MCFA non-aerosolized (40.0 CT) and aerosolized (39.0 CT) treatments did result in a greater reduction of detectable genetic material compared to Sal CURB (37.3 CT) over the 7 days ($P < 0.05$).

The effect of day resulted in an increase of CT values from 29.5 to 34.6 over the 4-day experiment, with each of the analysis days being significant from one another ($P < 0.0001$; Table 2). The increase in CT value also indicates that there is less detectable genetic material present.

The effect of treatment on PEDV was also significant and the resulting CT values varied from treatment to treatment ($P < 0.0001$; Table 3). The treatments that resulted in the highest CT values, or less detectable genetic material, were the MCFA blends (non-aerosolized and aerosolized), caproic acid, caprylic acid, and Sal CURB resulting

in significant differences of 6.9, 6.3, 5.8, 5.0, and 3.0 CT, respectively compared to the PEDV positive control ($P < 0.05$). The FRA C12, CWG, soy oil, canola oil, PKO, and CO did not seem to have the same effect as the treatments mentioned above as they were statistically similar to the untreated control ($P > 0.05$).

Bioassay Results

As expected Sal CURB resulted in a negative bioassay along with 1% MCFA mixture, 0.66% caproic, 0.66% caprylic, and 0.66% capric acids (Table 4). In both instances the PEDV positive treatments at day 0 and 1 both resulted in positive bioassays along with each of the other day 1 treatments. However, it is important to point out that the coconut oil treatment was not deemed positive until the last day of the bioassay.

In summary, time, Sal CURB, 1% MCFA, 0.66% caproic, 0.66% caprylic, and 0.66% capric acids enhance the RNA degradation of PEDV in swine feed such that infectivity was prevented. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially-available formaldehyde product in the complete swine diet at 1% inclusion and as individual fatty acids.

Table 1. Effect of treatment × day post inoculation on PEDV detection using RT-PCR[1,2]

Item	Day 0	Day 1	Day 3	Day 7	SEM	$P =$
PEDV positive	28.3[wvxyz]	29.7[rstuv]	31.3[nopq]	32.7[klmn]	0.5239	0.0002
Sal CURB[3]	28.7[uvwxy]	33.0[jklm]	35.0[fgh]	37.3[cd]		
1% MCFA (aero)	33.3[ijkl]	36.3[def]	38.3[bc]	39.0[ab]		
1% MCFA (non-aero)	34.3[ghij]	38.3[bc]	37.0[cde]	40.0[a]		
0.66 % Caproic	33.7[hijk]	35.0[fgh]	36.3[def]	37.0[cde]		
0.66% Caprylic	34.3[ghij]	35.7[efg]	38.0[bc]	37.3[cd]		
0.66% Capric	29.3[stuvw]	30.7[opqrs]	34.0[ghij]	35.3[fg]		
0.66 % Lauric	28.3[vwxyz]	30.7[opqrs]	32.7[klmn]	34.7[ghi]		
1% Capric:Lauric	29.0[tuvwx]	31.7[mnop]	34.3[ghij]	34.3[ghij]		
0.3% FRA C12[4]	28.0[wxyz]	30.7[opqrs]	31.7[mnop]	33.7[hijk]		
1% Choice white grease	28.3[vwxyz]	30.0[qrstu]	30.7[opqrs]	32.0[lmno]		
1% Soy	27.7[xyz]	30.0[qrstu]	30.3[pqrst]	32.0[lmno]		
1% Canola	27.0[z]	30.7[opqrs]	31.0[opqr]	31.7[mnop]		
1% Palm kernel oil	27.7[xyz]	30.0[qrstu]	31.0[opqr]	33.0[klmn]		
2% Palm kernel oil	27.3[yz]	29.7[rstuv]	30.3[pqrst]	33.0[jklm]		
1% Coconut oil	28.0[wxyz]	30.3[pqrst]	31.3[nopq]	32.7[klmn]		
2% Coconut oil	27.3[yz]	29.3[stuvw]	29.7[rstuv]	32.7[klmn]		

[1] A total of 204 samples were used for the analysis with each treatment represented by a mean of N=3.
[2] Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.
[3] Kemin Industries, Des Moines, IA.
[4] Framelco, Raamsdonksveer, Netherlands.
[ab] Means within a row lacking a common superscript differ.

Table 2. Main effect of day post-inoculation on detection of PEDV by qRT-PCR[1]

	Day					
Item	0	1	3	7	SEM	$P =$
CT value[2]	29.5[a]	31.9[b]	33.1[c]	34.6[d]	0.13	<0.0001

[1] A total of 204 samples were used for the analysis with each day represented by a mean of N=51.

[2] Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.

[ab] Means within a row lacking a common superscript differ.

Table 3. Main effect of treatment prior to PEDV-inoculation on PEDV detection using RT-PCR[1,2]

Item	CT Value	SEM	$P =$
PEDV positive	30.5[gh]	0.262	<0.0001
Sal CURB[3]	33.5[d]		
1% MCFA (aero)	36.8[ab]		
1% MCFA (non aero)	37.4[a]		
0.66 % Caproic	35.5[b]		
0.66% Caprylic	36.3[c]		
0.66% Capric	32.4[e]		
0.66 % Lauric	31.6[f]		
1% Capric:Lauric	32.3[e]		
0.3% FRA C12[4]	31.0[fg]		
1% Choice white grease	30.3[hi]		
1% Soy	30.0[hi]		
1% Canola	30.1[hi]		
1% Palm kernel oil	30.3[ghi]		
2% Palm kernel oil	30.1[hi]		
1% Coconut oil	30.6[gh]		
2% Coconut oil	29.8[i]		

[1] A total of 204 samples were used for the analysis with each treatment represented by a mean of N=12.

[2] Cycle threshold required to detect the genetic material. A higher CT value means less genetic material present.

[3] Kemin Industries, Des Moines, IA.

[4] Framelco, Raamsdonksveer, Netherlands.

[ab] Means within a row lacking a common superscript differ.

Table 4. Effects of medium chain fatty acids, Sal CURB, and fat source on PEDV infectivity measured by pig fecal swabs and cecum content by qRT-PCR analysis[1]

		PEDV N-gene Real Time-PCR, cycle threshold (CT)					
			Fecal swabs				Cecum contents[5]
Item	Feed CT[3]	0 dpi[2]	2 dpi	4 dpi	6 dpi	7 dpi	7 dpi
d 0							
PEDV negative	> 40.0	---[4]	---	---	---	---	> 45.0
PEDV positive	28.3	---	-- +	+++	+++	+++	22.2
d 1							
PEDV positive	29.7	---	- ++	+++	+++	+++	20.9
Sal CURB[6]	33.0	---	---	---	---	---	> 45.0
1% MCFA (non-aero)	38.3	---	---	---	---	---	> 45.0
0.66 % Caproic	35.0	---	---	---	---	---	> 45.0
0.66% Caprylic	35.7	---	---	---	---	---	> 45.0
0.66% Capric	30.7	---	---	---	---	---	> 45.0
0.66 % Lauric	30.7	---	---	+++	+++	+-+	28.4
0.3% FRA C12[7]	30.7	---	---	+++	+++	+++	30.2
1% Choice white grease	30.0	---	---	+++	+++	+++	15.3
1% Soybean oil	30.0	---	---	+++	+++	+++	24.0
1% Canola oil	30.7	---	---	+++	+++	+++	20.3
1% Palm kernel oil	30.0	---	---	+++	+++	+++	22.1
1% Coconut oil	30.3	---	---	---	---	+-+	42.1

[1] An initial tissue culture containing 10^6 $TCID_{50}$/mL PEDV was diluted to 10^5 $TCID_{50}$/mL PEDV. 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 day and treatment were collected and diluted in PBS. 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 age.

[2] Day post inoculation.

[3] A cycle threshold (CT of > 40) was considered negative for presence of PEDV RNA. Feed CT analysis was carried out at Kansas State University.

[4] In each instance a (−) signals a negative pig in the bioassay and a (+) represents a positive fecal swab in the bioassay. Each day post inoculation within each treatment has three symbols within each row and column which represents one of the three pigs in each treatment.

[5] A cycle threshold (CT of > 45) was considered negative for presence of PEDV RNA. Cecum content analysis was carried out at Iowa State University.

[6] Kemin Industries, Des Moines, IA.

[7] Framelco, Raamsdonksveer, Netherlands.