

Title: Evaluating alternative chemical mitigants to prevent PEDV recontamination of swine feed and ingredients - **NPB #:** 15-062

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Industry 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 MCFA levels below 2% inclusion is unknown. The objective of this experiment was to evaluate if a 1% inclusion of MCFA is as effective at PEDV mitigation as a 2% inclusion or formaldehyde in swine feed and spray-dried animal plasma (SDAP). Treatments were arranged in a $4 \times 2 \times 7$ plus 2 factorial with 4 chemical treatments: 1) PEDV positive with no chemical treatment, 2) 0.325% commercial formaldehyde, 3) 1% MCFA, and 4) 2% MCFA. The 2 matrices were: 1) complete swine diet and 2) SDAP; with 7 analysis days: 0, 1, 3, 7, 14, 21, and 42 post inoculation; and 1 treatment each of PEDV negative untreated feed and plasma. Matrices were first chemically treated, then inoculated with PEDV, stored at room temperature until being analyzed by RT-qPCR. The analyzed values represent threshold cycle (CT), at which a higher CT value represents less detectable RNA. All main effects and interactions were significant ($P < 0.009$). Feed treated with MCFA, regardless of inclusion level, had fewer ($P < 0.05$) detectable viral particles than feed treated with formaldehyde. However, the SDAP-treated with either 1% or 2% MCFA had similar ($P > 0.05$) concentrations of detectable PEDV RNA as the untreated SDAP, while the SDAP treated with formaldehyde had fewer detectable viral particles ($P < 0.05$). The complete feed had a lower ($P < 0.05$) quantity of PEDV RNA than SDAP (39.5 vs. 35.0 for feed vs. SDAP, respectively) ($P < 0.05$). Analysis day also decreased ($P < 0.05$) the quantity of detectable viral particles from d 0 to 42, (33.2 vs. 44.0, respectively). In summary, time, formaldehyde, and MCFA all appear enhance RNA degradation of PEDV in swine feed and ingredients; however, their effectiveness varies within matrix. The 1% inclusion level of MCFA was as effective as 2% in complete feed, but neither were effective at reducing the magnitude of PEDV RNA in SDAP.

Keywords: PEDV, medium chain fatty acids, feed matrix, swine

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Scientific 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 MCFA levels below 2% inclusion is unknown. The objective of this experiment was to evaluate if a 1% inclusion of MCFA is as effective at PEDV mitigation as a 2% inclusion or formaldehyde in swine feed and spray-dried animal plasma (SDAP). Treatments were arranged in a $4 \times 2 \times 7$ plus 2 factorial with 4 chemical treatments: 1) PEDV positive with no chemical treatment, 2) 0.325% commercial formaldehyde, 3) 1% MCFA, and 4) 2% MCFA. The 2 matrices were: 1) complete swine diet and 2) SDAP; with 7 analysis days: 0, 1, 3, 7, 14, 21, and 42 post inoculation; and 1 treatment each of PEDV negative untreated feed and plasma. Matrices were first chemically treated, then inoculated with PEDV, stored at room temperature until being analyzed by RT-qPCR. The analyzed values represent threshold cycle (CT), at which a higher CT value represents less detectable RNA. All main effects and interactions were significant ($P < 0.009$). Feed treated with MCFA, regardless of inclusion level, had fewer ($P < 0.05$) detectable viral particles than feed treated with formaldehyde. However, the SDAP-treated with either 1% or 2% MCFA had similar ($P > 0.05$) concentrations of detectable PEDV RNA as the untreated SDAP, while the SDAP treated with formaldehyde had fewer detectable viral particles ($P < 0.05$). The complete feed had a lower ($P < 0.05$) quantity of PEDV RNA than SDAP (39.5 vs. 35.0 for feed vs. SDAP, respectively) ($P < 0.05$). Analysis day also decreased ($P < 0.05$) the quantity of detectable viral particles from d 0 to 42, (33.2 vs. 44.0, respectively). In summary, time, formaldehyde, and MCFA all appear enhance RNA degradation of PEDV in swine feed and ingredients; however, their effectiveness varies within matrix. The 1% inclusion level of MCFA was as effective as 2% in complete feed, but neither were effective at reducing the magnitude of PEDV RNA in SDAP.

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 (Dee et al., 2014). Because of the potential viral spread by feed and ingredients, reduction techniques such as chemical treatments have been used to combat the virus. Many chemical treatments have been used to mitigate the virus, but formaldehyde and Medium Chain Fatty Acids (MCFA) seem to have the greatest reduction of the virus within feed and ingredients. Formaldehyde has shown to be effective at the approved rate of addition (37% formaldehyde used in animal feed at rate of 5.4 lb per ton), and MCFA at 2% wt/wt in the feed or ingredient (Cochrane et al., 2015; Dee et al., 2015; 21 CFR § 573.460). However, the efficacy of MCFA levels below 2% inclusion is unknown.

Objective

Evaluate if a 1% inclusion of MCFA is as effective at PEDV mitigation as a 2% inclusion or formaldehyde in swine feed and spray-dried animal plasma (SDAP).

Materials and Methods

In order to evaluate the use of chemical treatments on PEDV survival, a corn-soybean meal-based swine diet manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan Kansas, and spray-dried animal plasma were utilized. The feed matrices were first chemically treated before inoculation with PEDV in order to mimic post-processing contamination.

Chemical Treatment

In order to evaluate the chemical treatments a $4 \times 2 \times 7$ plus 2 factorial was utilized. The four chemical treatments; 1) positive control with PEDV and no chemical treatment, 2) 0.3% Sal CURB; Kemin Industries, Des Moines, IA, 3) 1% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1; Sigma Aldrich, St. Louis, MO] (aerosolized), and 4) 2% medium chain fatty acid blend [caproic, caprylic, and capric acids; 1:1:1] (aerosolized). These treatments were applied to 2 feed matrices 1) corn soybean meal-based swine

diet and 2) spray dried animal plasma, and evaluated on 7 analysis days (d 0, 1, 3, 7, 14, 21, and 42 post inoculation). There was also 1 treatment each of PEDV negative untreated feed and plasma, which acted as controls.

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

Once 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 mixer was 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 Virus 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). The stock virus titer contained 4.5×10^6 TCID₅₀/ml and was diluted to 10^5 TCID₅₀/ml.

Inoculation

The feed was inoculated using an appropriately sized pipet to allow even distribution of the virus within the feed and plasma. 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 TCID₅₀/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, 7, 14, 21, and 42 days post treatment 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 collected 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. A total of 60 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 20 rooms (60 pigs) were assigned to treatment groups with 2 negative control rooms and 18 challenge rooms. During bioassays, rectal swabs were collected on d -2, 0, 2, 4, 6, and 7 days 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.⁹

Statistical Analysis

Data of the main effects day, treatment, feed matrix, and all associated interactions 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 (see attached tables)

qRT-PCR Results

All main effects and interactions were highly significant ($P < 0.0037$). Overall, Sal CURB and MCFA both differed from the control ($P < 0.05$). Sal CURB was the most effective chemical treatment (CT = 38.3), followed by the 2% MCFA (CT = 38.2), and 1% MCFA (CT = 38.0), all of which reduced ($P < 0.05$) the quantity of detectable PEDV nucleic acid compared to the PEDV positive untreated control (CT = 34.6) as detected by qRT-PCR (Table 1).

Significant differences were also observed between each of the feed matrixes ($P < 0.0001$). Overall, complete diet had the greater PEDV CT, or less genetic material present (CT = 39.5), compared to the spray-dried animal plasma (CT = 35.0; Table 2).

Time also affected PEDV concentration detected by RT-PCR, with d 0 and 1 being statistically similar (33.2 vs. 34.3 CT, respectively; $P > 0.05$), but lower ($P < 0.05$) than d 3 (CT = 35.9 Table 3). The CT increased over time during d 3, 7, 14, 21, and 42 ($P < 0.05$; 35.9, 36.5, 38.0, and 39.0, and 44.0 respectively).

Interactions are presented graphically and provide more relevant results regarding the effects of specific chemical mitigants in the complete diet and spray-dried animal plasma over time. The PEDV CT in the untreated control of the complete diet increased in a linear fashion from d 0-42 (Figure 1). The chemical treatments all had a greater decrease in detectable PEDV RNA at each analysis day than the untreated control. In the complete swine diet, the MCFA treatments regardless of concentration were the most effective overall.

The PEDV CT in the untreated control of the spray-dried animal plasma had the same trend for both MCFA treatments (Figure 2). However, the commercial formaldehyde product was highly successful at mitigating PEDV according to qRT-PCR in spray-dried animal plasma compared to the MCFA treatments.

Bioassay Results

The bioassay provided a more in depth look at each of the chemical treatments as to which treatments led to no infection in the animals. In the complete feed, the only treatment that led to PEDV positive pigs was the day 0 PEDV positive feed with no chemical treatments (Table 4). However, in the spray-dried animal plasma Sal CURB was the only treatment that led to a negative bioassay on d 3 (Table 5). On d 21 the Sal CURB, 1% MCFA, and PEDV positive untreated control all led to negative bioassays with the 2% MCFA treatment producing a positive bioassay 4 days' post inoculation (Table 5).

Conclusion

In summary, time, Sal CURB, and MCFA enhance the RNA degradation of PEDV in swine feed and ingredients, but their effectiveness varies within matrix. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially available formaldehyde product in the complete swine diet at 1% inclusion.

References

- 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.
- Cochrane et al., 2015. Evaluating chemical mitigation of Porcine Epidemic Diarrhea Virus in Swine Feed and Ingredients. *Kansas State University Swine Day. KS Agric. Exp. Stn. Res.* 1:7.
- 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.

Dee et al., 2015. An evaluation of porcine epidemic diarrhea virus survival in individual feed ingredients in the presence or absence of a liquid antimicrobial. *Porcine Health Management*. 1:9. doi 10.1186/s40813-015-0003-0.

Formaldehyde. 2003. 21 CFR § 573.460.

I. Tables and Figures

Table 1. Main effect of treatment on detection of PEDV by qRT-PCR¹

Item	PEDV pos.	Sal CURB	1% MCFA	2% MCFA	SEM	<i>P</i> =
CT value ²	34.6 ^b	38.3 ^a	38.0 ^a	38.2 ^a	0.43	<0.0001

¹ A total of 168 samples were used for the analysis with each treatment represented by a mean of N=42.

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 2. Main effect of feed matrix post inoculation on detection of PEDV by qRT-PCR¹

Item	Feed	SDAP	SEM	<i>P</i> =
CT value ²	39.5 ^a	35.0 ^b	0.43	<0.0001

¹ A total of 168 samples were used for the analysis with each day represented by a mean of N=84.

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 day post inoculation on detection of PEDV by qRT-PCR¹

Item	Day							SEM	<i>P</i> =
	0	1	3	7	14	21	42		
CT value ²	33.2 ^e	34.3 ^e	35.9 ^d	36.5 ^{cd}	38.0 ^{bc}	39.0 ^b	44.0 ^a	0.13	<0.0001

¹ A total of 168 samples were used for the analysis with each day represented by a mean of N=24.

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.

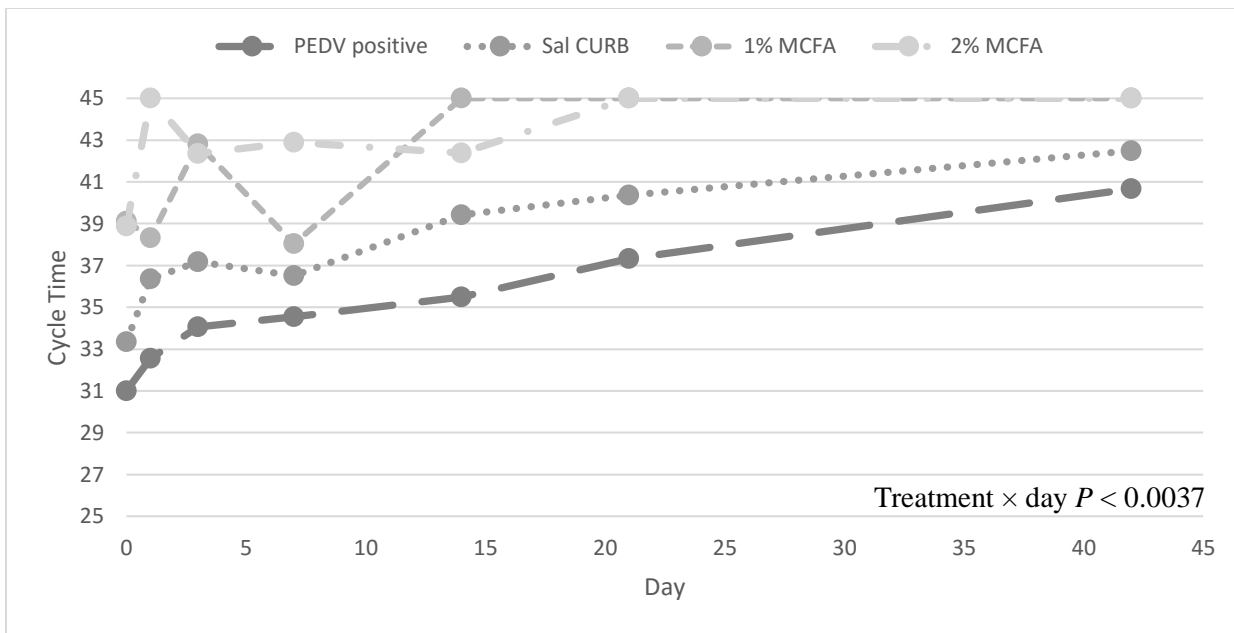


Figure 1. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated complete swine diet stored at room temperature.^{1,2}

¹Data was analyzed by PCR with each data point represented by N=3

²The higher the CT value, the less quantity of PEDV RNA genetic material is detected.

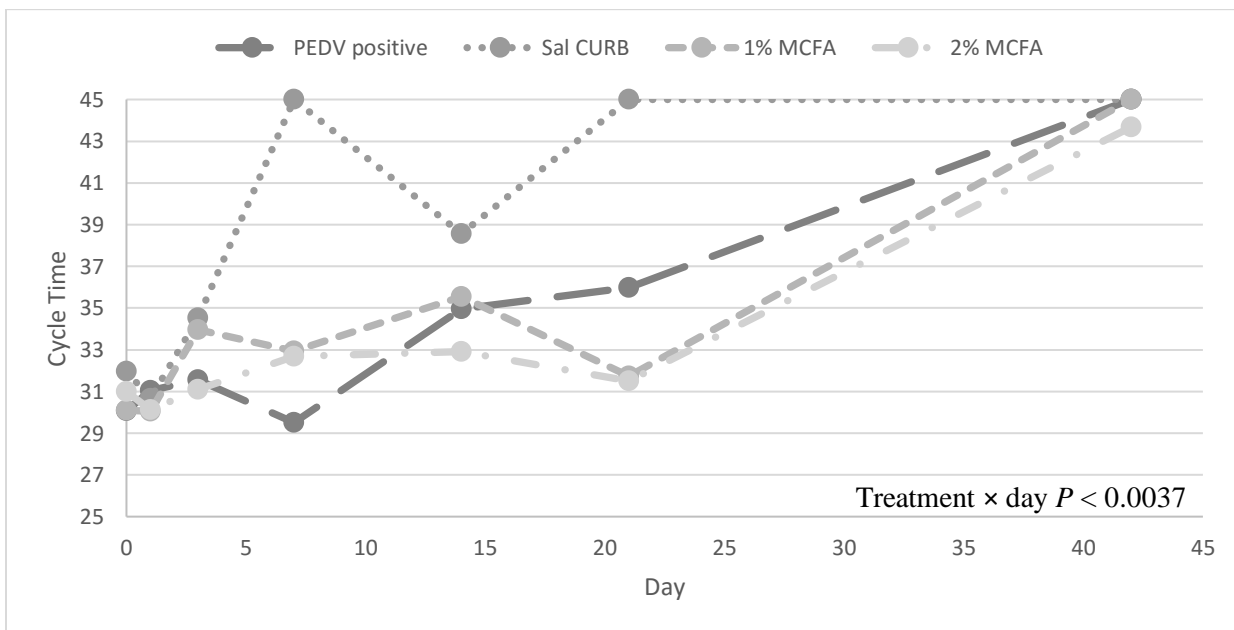


Figure 2. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated spray dried animal plasma stored at room temperature.^{1,2}

¹Data was analyzed by PCR with each data point represented by N=3

²The higher the CT value, the less quantity of PEDV RNA genetic material is detected.

Table 4. Effects of medium chain fatty acids and formaldehyde treatment of complete diet on porcine epidemic diarrhea virus (PEDV) detection from feed, pig fecal swabs and cecum contents¹

Item	PEDV N-gene Real Time-PCR, cycle threshold (Ct)						
	Feed CT	Fecal swabs					Cecum contents ⁵
		0 dpi ²	2 dpi	4 dpi	6 dpi	7 dpi	7 dpi
Unprocessed virus-free feed	> 45.0 ³	---	---	---	---	---	> 45.0
Day 0 inoculated feed	31.0	---	---	- + -	++ -	++ -	28.0
Day 3 inoculated feed	34.1	---	---	---	---	---	> 45.0
Day 3 Sal CURB	37.2	---	---	---	---	---	> 45.0
Day 3 1% MCFA	42.8	---	---	---	---	---	> 45.0
Day 3 2% MCFA	42.4	---	---	---	---	---	> 45.0
Day 21 inoculated feed	37.3	---	---	---	---	---	> 45.0
Day 21 Sal CURB	40.4	---	---	---	---	---	> 45.0
Day 21 1% MCFA	> 45.0	---	---	---	---	---	> 45.0
Day 21 2% MCFA	> 45.0	---	---	---	---	---	> 45.0

¹An initial tissue culture containing 10⁶ TCID₅₀/mL PEDV was diluted to 10⁵ TCID₅₀/mL PEDV. Each treatment was inoculated with the 10⁵ TCID₅₀/mL PEDV resulting in 10⁴ TCID₅₀/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). Pigs were inoculated at d 12 age.

²Day post inoculation.

³A cycle threshold (Ct) of >45 was considered negative for presence of PEDV RNA. Feed CT values were analyzed at Kansas State University.

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

⁵ Each cecum content value represents the mean of 3 pigs per treatment and was analyzed at Iowa State University.

Table 5. Effects of medium chain fatty acids and formaldehyde treatment of spray dried porcine plasma on porcine epidemic diarrhea virus (PEDV) detection from plasma, pig fecal swabs and cecum contents¹

Item	PEDV N-gene Real Time-PCR, cycle threshold (Ct)						
	Plasma CT	Fecal swabs					Cecum contents
		0 dpi ²	2 dpi	4 dpi	6 dpi	7 dpi	7 dpi ⁵
Unprocessed virus-free feed	> 45.0 ³	--- ⁴	---	---	---	---	> 45.0
Day 0 inoculated plasma	30.1	---	+++	+++	+++	+++	27.8
Day 3 inoculated plasma	31.6	---	+++	+++	+++	+++	29.8
Day 3 Sal CURB	34.5	---	---	---	---	---	> 45.0
Day 3 1% MCFA	34.0	–	+++	+++	+++	+++	30.4
Day 3 2% MCFA	31.1	–	+++	+++	+++	+++	29.4
Day 21 inoculated plasma	36.0	---	---	---	---	---	> 45.0
Day 21 Sal CURB	> 45.0	---	---	---	---	---	> 45.0
Day 21 1% MCFA	31.7	---	---	---	---	---	> 45.0
Day 21 2% MCFA	31.5	---	---	- + -	+++	+++	31.3

¹An initial tissue culture containing 10⁶ TCID₅₀/mL PEDV was diluted to 10⁵ TCID₅₀/mL PEDV. Each treatment was inoculated with the 10⁵ TCID₅₀/mL PEDV resulting in 10⁴ TCID₅₀/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, each value represents the mean of 3 pigs per treatment. Pigs were inoculated at d 12 age.

²Day post inoculation.

³A cycle threshold (Ct) of >45 was considered negative for presence of PEDV RNA.

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

⁵Each cecum content value represents the mean of 3 pigs per treatment and was analyzed at Iowa State University.