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Quantifying Medium Chain Fatty Acid Mitigation Activity Over Time against Porcine Epidemic Diarrhea Virus in Nursery Pig Diets

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Quantifying Medium Chain Fatty Acid Mitigation Activity Over Time against Porcine Epidemic Diarrhea Virus in Nursery Pig Diets

Abstract

Medium chain fatty acids (MCFA) are six to twelve carbon length molecules that have shown significant promise as potential mitigants of biological hazards in feed and feed ingredients. The use of residual duration of activity approaches, such as MCFA, have significant advantages compared to point-in-time mitigation strategies. The primary advantage of MCFA is the ability to mitigate the risks generated by post-processing contamination; however, the duration of mitigation activity has not been established. Therefore, the objective of this experiment was to characterize the mitigation properties of MCFA-treated swine feed 40 d following feed manufacturing. Treatments (n = 8) consisted of a dose response including 0, 0.25, 0.50, 1.0, and 1.5% dietary inclusion of a MCFA blend (1:1:1 ratio C6, C8, and C10) as well as 0.5% C6 alone, 0.5% C8 alone, or 0.5% C10 alone. Following feed manufacturing, feed was stored in bags at barn temperature and humidity for 40 d (June to July 2017). Following sampling after storage, subsamples were placed in separate high-density polyethylene bottles and inoculated with Porcine Epidemic Diarrhea Virus (PEDV) to achieve a final titer of 10^4 $TCID_{50}$/g. Separate sample bottles were analyzed on d 0 and 3 post-inoculation. A significant treatment × day interaction ($P < 0.001$) was observed, where the cycle threshold (Ct) numerically increased over time in select treatments, and was numerically reduced in others. Means separation, adjusted to control experiment-wise error rate, did not indicate evidence of a difference within treatment among days of analysis ($P > 0.05$) for any of the eight treatments. When evaluating increasing inclusion of MCFA blend, an inclusion level × day interaction was observed (quadratic, $P = 0.023$), where PEDV Ct values increased (quadratic, $P = 0.001$) on d 0 with increasing levels of MCFA blend inclusion also increased on d 3 (linear, $P < 0.001$). On d 0 post-inoculation, the addition of C6, C8, or C10 alone resulted in significantly greater Ct values compared to no supplemented MCFA ($P < 0.05$). The addition of 0.5% C6 and 0.5% C8 did not change Ct value ($P > 0.05$) compared to 0.5% MCFA blend; however, adding 0.5% C10 resulted in a lower Ct value ($P < 0.05$) compared to 0.5% MCFA blend. On d 3 post-inoculation, the addition of 0.5% C6 or 0.5% C10 resulted in greater Ct values compared to control ($P < 0.05$), whereas, no improvement was observed with 0.5% C8 compared to control ($P > 0.05$). The addition of 0.5% MCFA blend resulted in insufficient evidence of difference in Ct values compared to adding individual MCFA ($P > 0.05$).

In summary, treatment of feed with medium chain fatty acids retains mitigation properties for a significant period of time following feed manufacturing. Although we did not assess infectivity through bioassay, the data herein suggest a residual duration mitigation potential for MCFA well beyond feed manufacturing. Additional research characterizing the duration of activity beyond one point in time following feed manufacturing is warranted.

Keywords

medium chain fatty acid, mitigation, PEDV

Cover Page Footnote
Appreciation is expressed to Dr. Dick Hesse, Dr. Jianfa Bai, Elizabeth Poulsen, and Joe Anderson for technical support and laboratory use.

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K-STATE Research and Extension

Swine Day 2017

Quantifying Medium Chain Fatty Acid Mitigation Activity Over Time against Porcine Epidemic Diarrhea Virus in Nursery Pig Diets[1]

J.T. Gebhardt, J.C. Woodworth, M.D. Tokach, J.M. DeRouchey, R.D. Goodband, C.K. Jones, and S.S. Dritz[2]

Summary

Medium chain fatty acids (MCFA) are six to twelve carbon length molecules that have shown significant promise as potential mitigants of biological hazards in feed and feed ingredients. The use of residual duration of activity approaches, such as MCFA, have significant advantages compared to point-in-time mitigation strategies. The primary advantage of MCFA is the ability to mitigate the risks generated by post-processing contamination; however, the duration of mitigation activity has not been established. Therefore, the objective of this experiment was to characterize the mitigation properties of MCFA-treated swine feed 40 d following feed manufacturing. Treatments (n = 8) consisted of a dose response including 0, 0.25, 0.50, 1.0, and 1.5% dietary inclusion of a MCFA blend (1:1:1 ratio C6, C8, and C10) as well as 0.5% C6 alone, 0.5% C8 alone, or 0.5% C10 alone. Following feed manufacturing, feed was stored in bags at barn temperature and humidity for 40 d (June to July 2017). Following sampling after storage, subsamples were placed in separate high-density polyethylene bottles and inoculated with Porcine Epidemic Diarrhea Virus (PEDV) to achieve a final titer of 10^4 $TCID_{50}$/g. Separate sample bottles were analyzed on d 0 and 3 post-inoculation. A significant treatment × day interaction ($P < 0.001$) was observed, where the cycle threshold (Ct) numerically increased over time in select treatments, and was numerically reduced in others. Means separation, adjusted to control experiment-wise error rate, did not indicate evidence of a difference within treatment among days of analysis ($P > 0.05$) for any of the eight treatments. When evaluating increasing inclusion of MCFA blend, an inclusion level × day interaction was observed (quadratic, $P = 0.023$), where PEDV Ct values increased (quadratic, $P = 0.001$) on d 0 with increasing levels of MCFA blend inclusion also increased on d 3 (linear, $P < 0.001$). On d 0 post-inoculation, the addition of C6, C8, or C10 alone resulted in significantly greater Ct values compared to no supplemented MCFA ($P < 0.05$). The addition of 0.5% C6 and 0.5% C8 did not change Ct value ($P > 0.05$) compared to 0.5% MCFA blend; however, adding 0.5%

[1] Appreciation is expressed to Dr. Dick Hesse, Dr. Jianfa Bai, Elizabeth Poulsen, and Joe Anderson for technical support and laboratory use.

[2] Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

C10 resulted in a lower Ct value ($P < 0.05$) compared to 0.5% MCFA blend. On d 3 post-inoculation, the addition of 0.5% C6 or 0.5% C10 resulted in greater Ct values compared to control ($P < 0.05$), whereas, no improvement was observed with 0.5% C8 compared to control ($P > 0.05$). The addition of 0.5% MCFA blend resulted in insufficient evidence of difference in Ct values compared to adding individual MCFA ($P > 0.05$).

In summary, treatment of feed with medium chain fatty acids retains mitigation properties for a significant period of time following feed manufacturing. Although we did not assess infectivity through bioassay, the data herein suggest a residual duration mitigation potential for MCFA well beyond feed manufacturing. Additional research characterizing the duration of activity beyond one point in time following feed manufacturing is warranted.

Introduction

There is significant focus on solutions to minimize the risk of disease transmission via feed and feed ingredients after PEDV introduction in North America. One solution, which has shown significant promise, is the use of medium chain fatty acids (MCFA), which are free fatty acids consisting of 6, 8, 10, or 12 carbon molecules. These molecules have shown efficacy at reducing both the quantity of detectable genetic material and infectivity characteristics, with the 6, 8, and 10 carbon length fatty acids seeming to be most effective.[3,4] When summarizing various mitigation options for biological agents in feed and feed ingredients, strategies can be categorized into one of two approaches. Point-in-time mitigation involves deactivation of an agent at a particular moment, such as thermal processing or irradiation. However, there is no residual mitigation activity, and the feed or ingredient is susceptible to re-contamination post-processing. Another approach to biological agent mitigation includes the use of compounds with residual mitigation activity, including feed additives, such as MCFA, formaldehyde, essential oils, dietary acidifiers, etc. However, to date, no research has been conducted quantifying the residual mitigation properties of MCFA beyond one day post feed manufacturing. To fully understand the risk mitigation value of such feed additives, it is essential to understand the appropriate time frame in which biological agent mitigation can occur in complete swine feed following manufacturing with MCFA. Therefore, the objective of this experiment was to determine the mitigation characteristics of complete swine feed treated with MCFA when inoculated with PEDV 40 d following feed manufacturing.

[3] Cochrane, R. A., S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, M. Saensukjaroephon, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. F. Bai, Q. Chen, J. Zhang, P. C. Gauger, R. J. Derscheid, R. G. Main, and C. K. Jones. 2017. Assessing the effects of medium chain fatty acids and fat sources on PEDV RNA stability and infectivity. J. Anim. Sci. 95 (Suppl. 2):196 (Abstr.).

[4] Cochrane, R. A., M. Saensukjaroenphon, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. F. Bai, Q. Chen, J. Zhang, P. C. Gauger, R. Main, and C. K. Jones. 2016. Evaluating the inclusion level of medium chain fatty acids to reduce the risk of PEDV in feed and spray-dried animal plasma. J. Anim. Sci. 94 (Suppl 2):50. doi:10.2527/msasas2016-107.

Procedures

General

The swine diet (Table 1) used in this experiment was manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS, in 8 separate treatments. Dose response treatments included the 0, 0.25, 0.50, 1.0, and 1.5% addition of a MCFA blend (1:1:1 ratio C6, C8, and C10) with additional treatments of 0.5% C6 alone, 0.5% C8 alone, or 0.5% C10. The MCFA treatments were added at the expense of soybean oil. The MCFA were guaranteed ≥ 98% purity, and were sourced individually (Sigma Aldrich, St. Louis, MO) and manually blended for the appropriate treatments. Following feed manufacturing, feed was stored in 50 lb paper bags at barn temperature and humidity at the K-State Segregated Early Wean facility for 40 d (June – July 2017). Following storage, samples were collected from multiple bags per treatment and subsampled using a riffle-splitter. Six, 22.5 g samples of each treatment were placed in separate 250 mL high density polyethylene (HDPE) bottles (Thermo Fisher Scientific, Waltham, MA) to be inoculated with PEDV and analyzed on two sampling days post laboratory inoculation (d 0 and 3) with 3 replications of each sampling day and treatment combination. In addition, 22.5 g of feed without MCFA was added to three separate bottles as control samples which were not inoculated with PEDV and were analyzed along with the d 0 inoculated bottles. Complete diet samples used in laboratory analysis of MCFA content were collected following feed manufacturing using a feed probe to collect samples, subsampled, and submitted to University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) for MCFA composition analysis using gas chromatography.

Inoculation

Inoculation was carried out at the Kansas State University College of Veterinary Medicine Virology Laboratory. The viral inoculum was cell culture derived USA/IN/2013/19338, passage 9 and had an initial concentration of 10^5 $TCID_{50}$/mL. Inoculation occurred by pipetting 2.5 mL of diluted viral inoculum into each bottle containing 22.5 g feed matrix, resulting in an inoculated feed matrix with a viral concentration of 10^4 $TCID_{50}$/g of feed matrix. Following the addition of the viral inoculum to each bottle, the bottles were lightly shaken in a circular pattern for approximately five seconds, after which, each bottle was vigorously hand shaken for approximately 10 s to mix the virus evenly within each bottle. The three negative control bottles had 2.5 mL of PBS added to each bottle as a sham inoculation following similar procedures to the viral inoculation. Samples were stored at room temperature until analysis performed on appropriate day post inoculation.

Real-Time PCR Analysis

Separate bottles were analyzed on d 0 and 3 post-laboratory inoculation. On each day of analysis, 100 mL phosphate buffered saline (PBS; pH 7.4 1X, Life Technologies, Grand Island, NY) was added to each bottle predetermined for analysis on that day. Bottles were shaken for approximately 10 s, at which point they could settle overnight at 39.2°F. The following day, supernatant was pulled and aliquoted for further analysis.

A total of 2 aliquots from each sample bottle were collected and stored at -4°F until the conclusion of the trial, at which point qRT-PCR analysis was performed on one aliquot per sample bottle and the remaining sample was stored at -112°F.

The qRT-PCR was conducted at K-State Veterinary Diagnostic Laboratory Molecular Diagnostics Lab as previously described.[5] Briefly, 50 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 -4°F until assayed by qRT-PCR. Analyzed values represent the cycle threshold (Ct) at which virus was detected. A greater Ct value indicates more cycles must proceed until viral genetic material is detected, thus lower quantities of genetic material are present in the original sample.

Statistical Analysis

Treatments were arranged as an 8 × 2 factorial with the main effects of diet, treatment, and day post-laboratory inoculation, and their interaction on PEDV Ct values included in the model using individual sample bottle as the experimental unit. Means separation was performed using the LINES option, with the experiment-wise error rate set at $\alpha = 0.05$ using a Tukey-Kramer multiple comparisons adjustment. Additionally, increasing MCFA blend supplementation was evaluated using linear × day post-inoculation and quadratic × day post-inoculation interactions. Linear and quadratic effects of increasing MCFA supplementation are not presented due to the presence of a significant quadratic level × day interaction. Results for the response criteria were considered significant at $P \leq 0.05$ and marginally significant from $P > 0.05$ to $P \leq 0.10$. All data were analyzed using PROC GLIMMIX (SAS Institute, Inc., Cary, NC).

Results and Discussion

Analysis of diet samples resulted in analyzed values closely matching expected values based on added MCFA concentrations (Table 2).

A significant treatment × day interaction ($P < 0.001$) was observed, where the Ct numerically increased over time in select treatments, and was numerically reduced in others. Means separation, adjusted to control experiment-wise error rate, did not indicate evidence of a difference within treatment between day of analysis ($P > 0.05$) for any of the eight treatments. Previous research evaluating PEDV inoculation in feed and feed ingredients would generally demonstrate an increase in PEDV Ct values over time following inoculation as the viral stability naturally degrades. Day post-inoculation did not have a significant impact on PEDV genetic material quantification ($P = 0.741$); however, there was significant evidence that at least one dietary treatment differed from

[5] Gebhardt, J. T., J. C. Woodworth, C. K. Jones, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, R. A. Cochrane, C. R. Stark, J. Bergstrom, P. C. Gauger, J. Bai, Q. Chen, J. Zhang, R. G. Main, and S. S. Dritz. 2016 Evaluating the Impact of VevoVitall and/or CRINA as Potential Porcine Epidemic Diarrhea Virus Mitigation Strategies as Determined by Polymerase Chain Reaction Analysis and Bioassay. *Kansas Agricultural Experiment Station Research Reports*: Vol. 2: Iss. 8. https://doi.org/10.4148/2378-5977.1281.

others ($P < 0.001$). When evaluating increasing MCFA blend, an inclusion level × day interaction was observed (quadratic, $P = 0.023$), where PEDV Ct values increased in a quadratic manner ($P < 0.001$) on d 0 with increasing levels of MCFA blend inclusion, but increased in a linear manner on d 3 (linear, $P < 0.001$). There was not sufficient evidence demonstrating a quadratic response on d 3 ($P = 0.745$), leading to the presence of an interaction. This difference in response among days is largely driven by an increase in Ct value ($P < 0.05$) between 0.25% MCFA blend inclusion and control treatments on d 0, whereas, there was not sufficient evidence of a difference between these treatments ($P > 0.05$) on d 3.

On d 0 post-inoculation, the addition of C6, C8, or C10 alone resulted in significantly greater Ct values compared to no supplemented MCFA ($P < 0.05$). Inclusion of 0.5% C6 or 0.5% C8 did not result in differences in Ct value ($P > 0.05$) compared to 0.5% MCFA blend; however, adding 0.5% C10 resulted in a lower Ct value ($P < 0.05$) compared to 0.5% MCFA blend, indicating C10 may not be as effective as C6 or C8 at mitigation of PEDV. On d 3 post-inoculation, the addition of 0.5% C6 or 0.5% C10 resulted in greater Ct values compared to control ($P < 0.05$), whereas, no improvement was observed with 0.5% C8 ($P > 0.05$). The addition of 0.5% MCFA blend resulted in no evidence of difference in Ct values compared to adding individual MCFA ($P > 0.05$).

In summary, treating feed with medium chain fatty acids retains mitigation properties for at least 40 d following feed manufacturing. Thus, the data herein demonstrate mitigants retain activity well beyond feed manufacturing; however, additional research is warranted characterizing the duration of activity beyond one point in time following feed manufacturing.

Table 1. Diet composition (as-fed basis)

Item	Swine nursery diet
Ingredient, %	
Corn	62.55
Soybean meal, 46.5% CP	31.60
Soybean oil	1.50
Calcium carbonate	1.00
Monocalcium phosphate, 21% P	1.15
Salt	0.60
L-Lys HCl	0.51
DL-Met	0.23
L-Thr	0.21
L-Trp	0.06
L-Val	0.14
Trace mineral premix	0.15
Vitamin premix	0.25
Phytase[2]	0.07
Hexanoic acid[3]	+/-
Octanoic acid[3]	+/-
Decanoic acid[3]	+/-
Total	100

continued

Table 1, *continued*. Diet composition (as-fed basis)

Item	Swine nursery diet
Calculated analysis[4]	
Standardized ileal digestible (SID) amino acids, %	
Lys	1.35
Ile:Lys	55
Leu:Lys	113
Met:Lys	37.3
Met and Cys:Lys	58.1
Thr:Lys	62.0
Trp:Lys	20.3
Val:Lys	70.1
Total Lys, %	1.49
ME, kcal/lb	1,518
NE, kcal/lb	1,127
SID Lys:ME, g/Mcal	4.03
SID Lys:NE, g/Mcal	5.43
CP, %	21.1
Ca, %	0.70
P, %	0.63
Available P, %	0.46
STTD P, %	0.50

[1] Diet was formulated to be fed to 25-50 lb BW nursery pigs.

[2] HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ).

[3] Sigma Aldrich (St. Louis, MO), guaranteed ≥ 98% purity added at the expense of soybean oil in appropriate treatments.

[4] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Table 2. Effect of medium chain fatty acid (MCFA) inclusion in swine complete feed on analyzed MCFA level and quantification of PEDV genetic material using qRT-PCR following inoculation[1]

	MCFA inclusion, %[1]							
		C6:C8:C10[2]				C6	C8	C10
Item	0	0.25	0.5	1.0	1.5	0.5	0.5	0.5
Analyzed MCFA, %[3]								
Hexanoic acid	0.02	0.07	0.15	0.24	0.50	0.41	0.02	0.01
Octanoic acid	0.01	0.06	0.15	0.29	0.55	0.04	0.37	0.01
Decanoic acid	0.02	0.07	0.18	0.32	0.62	0.02	0.01	0.49
Total MCFA[4]	0.05	0.21	0.48	0.85	1.67	0.47	0.40	0.51
PEDV PCR Feed Ct[5,6]								
0 dpi	27.1^{f}	$29.5^{c,d,e}$	$30.9^{b,c}$	$30.6^{b,c}$	32.4^{a}	$29.7^{c,d,e}$	$30.0^{c,d}$	$28.7^{d,e}$
3 dpi	$28.3^{e,f}$	$28.3^{e,f}$	$29.9^{c,d}$	$30.9^{b,c}$	$32.1^{a,b}$	30.5^{c}	$29.5^{c,d,e}$	$29.9^{c,d}$

[1] Complete swine feed was manufactured with varying medium chain fatty acid (MCFA) [hexanoic acid (C6), octanoic acid (C8), or decanoic acid (C10); Sigma Aldrich, St. Louis, MO] inclusion or a negative control (0) %.

[2] Consisted of a 1:1:1 blend of C6, C8, and C10.

[3] Complete diet samples were collected following feed manufacturing using a feed probe to collect samples, subsampled, and submitted to University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) to be analyzed in duplicate. Reported value represents mean of duplicate analysis.

[4] Sum of analyzed C6, C8, and C10.

[5] Each number is the mean of 3 samples of swine feed that were inoculated with porcine epidemic diarrhea virus (PEDV) 40 d following feed manufacturing with a final inoculated titer of 10^4 $TCID_{50}$/g feed. On d 0 and 3 post-inoculation, appropriate samples were analyzed for the presence of PEDV genetic material using qRT-PCR. Three bottles containing swine feed were not inoculated with PEDV as a negative control and analyzed on d 0, and qRT-PCR analysis did not detect genetic material using a threshold cutoff value of 45 cycles. A higher Ct value indicates a greater amount of PEDV genetic material present in the original sample.

[6] SEM = 0.27; Treatment × day, $P < 0.001$; Quadratic inclusion of MCFA blend × day, $P = 0.023$; Day 0 quadratic C6:C8:C10 inclusion, $P = 0.001$; Day 3 linear C6:C8:C10 inclusion, $P < 0.001$.

[a,b,c,d,e,f] Means lacking common superscript differ ($P < 0.05$). Tukey-Kramer multiple comparison adjustment applied in statistical model.

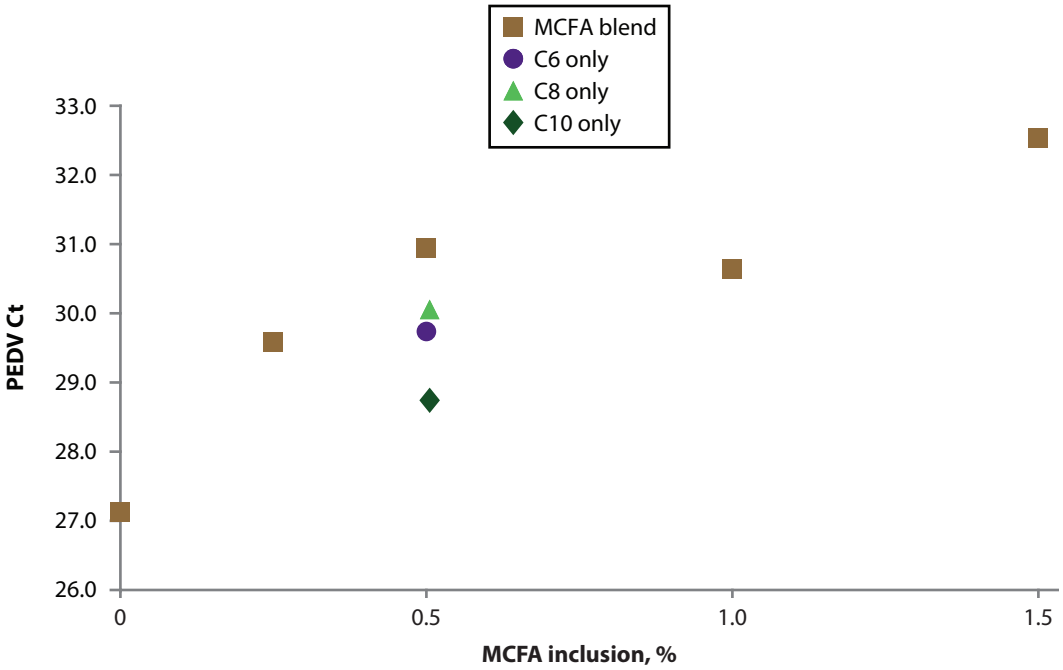

Figure 1. Effect of medium chain fatty acid (MCFA) inclusion on porcine epidemic diarrhea virus (PEDV) quantification on d 0 post-inoculation.

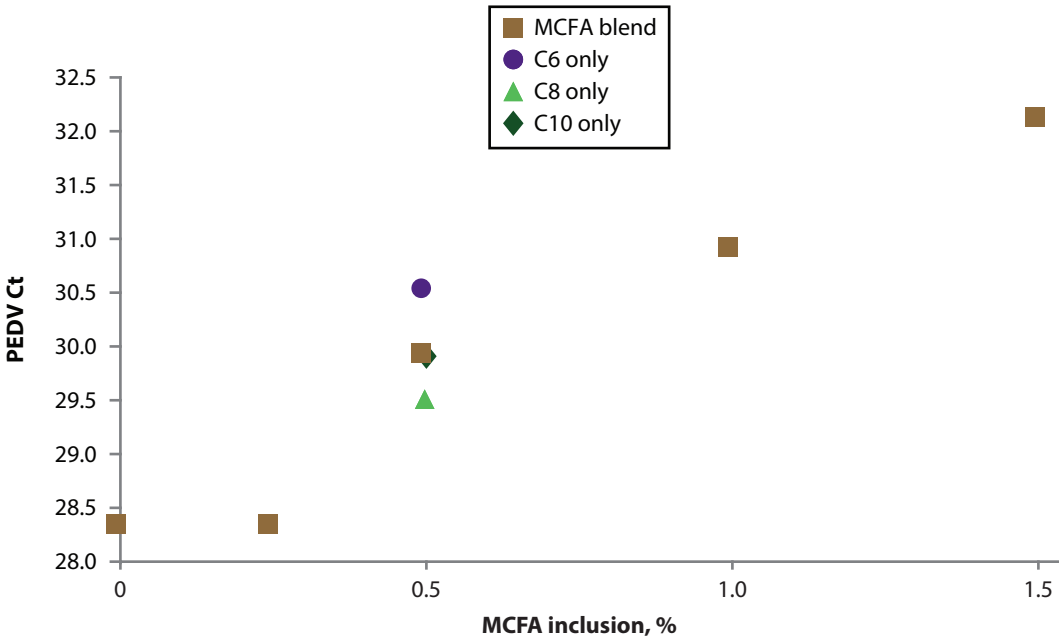

Figure 2. Effect of medium chain fatty acid (MCFA) inclusion on porcine epidemic diarrhea virus (PEDV) quantification on d 3 post-inoculation.