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Monoglyceride reduces viability of porcine epidemic diarrhoea virus in feed and prevents disease transmission to post-weaned piglets

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Abstract

Outbreaks of African swine fever virus (ASFv) and porcine epidemic diarrhoea virus (PEDv) have revealed the susceptibility of livestock to disease transmitted through feed. Several viruses, including PEDv, survive in feed and may introduce disease that causes significant morbidity and mortality. In 2013, PEDv, which causes severe diarrhoea and vomiting, reached North America after spreading for decades across Eurasia. The global exchange of ingredients has created demand for products that prevent disease transmission from feed. Formaldehyde-based products are highly effective at inactivating enveloped viruses when applied at 3.25 kg/t. Alternative products to formaldehyde, including carboxylic acids, essential oils and medium chain fatty acids (MCFAs), have exhibited mixed efficacy against PEDv and require application rates higher than formaldehyde. Amphiphilic molecules like MCFAs disrupt the bilayer-lipid membranes that protect viral nucleic acids through the formation of micelles. Monoglycerides form micelles at lower concentrations than MCFAs, which suggests they may be more potent against enveloped viruses. The potential efficacy of monoglycerides against enveloped viruses in feed led to the development and examination of an experimental monoglyceride blend. The proprietary monoglyceride blend significantly ($p < .0001$) reduced PEDv viability in vitro after application to feed at 1.5, 2.5 and 3.5 kg/t. The monoglyceride was tested in a natural feeding behaviour challenge model in piglets. The feed was contaminated with ice-blocks containing viable PEDv, and the piglets were exposed to PEDv through the feed bin for 20 days. At the end of the 20-day challenge period, all pigs were rectally swabbed and tested for PEDv by qPCR. In the untreated control group 54.8% of the piglets tested positive for PEDv, whereas none of the MCFA-treated feed (10 kg/t inclusion) transmitted PEDv. Strikingly, the monoglyceride-treated groups (1.5, 2.5 and 3.5 kg/t) all exhibited 100% protection from PEDv. These data support the use of this proprietary monoglyceride blend in mitigation and prevention of viral disease transmission to piglets from contaminated feed.

1 | INTRODUCTION

Lipids found in human milk, including medium chain fatty acids (MCFAs), defined as saturated fatty acids with C_6 to C_{12} hydrocarbon chains, and monoglycerides effectively inactivate enveloped viruses (Thormar et al., 1987). Several decades of research beginning in the 1800s document the efficacy of lipids against algae, bacteria, fungi, protozoa, and viruses (Yoon et al., 2018). Broad antimicrobial properties of MCFAs and related monoglycerides hold the promise of a next generation of antimicrobial agents with a low risk of pathogens developing resistance (Davies, 1996). The mechanism by which MCFAs and monoglycerides inactivate viruses and kill or inhibit bacteria is through disruption of the viral envelope and bacterial cell membranes, respectively (Yoon et al., 2018). The hydrophilicity of the carboxyl group paired with the hydrophobicity of the hydrocarbon tail makes fatty acids amphiphilic. The single-chain lipid amphiphile inserts into the lipid bilayer of bacteria causing strain on their structures. This strain forces remodelling processes that generate tubules or spherical buds in the lipid bilayer (Yoon et al., 2017).

Porcine epidemic diarrhoea virus (PEDv) is an enveloped single-stranded positive sense RNA virus that is part of the *Coronaviridae* family under the genus *Alphacoronavirus* (Wang et al., 2019). In 2013, PEDv escaped its endemic locations in Europe and Asia and spread rapidly across the United States killing an estimated 7 million pigs over a 12-month period (Niederwerder & Hesse, 2018). PEDv causes an enteric disease that largely affects neonatal piglets resulting in severe vomiting and diarrhoea, and spreads directly through faecal–oral route (Jung & Saif, 2015). The virus can also be carried into barns by trailers, farm workers' hands, boots, and clothes (Wang et al., 2019) as well as animal feed (Dee, Clement, et al., 2014; Dee et al., 2018, Dee et al., 2021). The identification of feed as a risk factor for introduction of PEDv led to research into identifying mechanisms that inactivate enveloped viruses in feed (Cottingim et al., 2017; Dee et al., 2020; Dee, Clement, et al., 2014; Dee, Neill, et al., 2014; Trudeau et al., 2016, 2017).

Several products from feed additive suppliers are available to inactivate viruses in feed and mitigate the risk of disease transmission from contaminated feed (Dee et al., 2020). Formaldehyde-based products are highly effective against viruses in feed when applied at >2.5 kg/t (Dee et al., 2020; Niederwerder et al., 2021). Alternatives to formaldehyde include carboxylic acids, MCFAs and essential oils, which are becoming more prevalent, especially in un-pelleted feed because thermal treatments have also been shown to inactivate PEDv (Cochrane et al., 2017). However, the efficacy of these alternative products is limited, and pelleting feed incurs additional costs to the producer. Recently, MCFAs were demonstrated to mitigate *Salmonella* Typhimurium (Cochrane et al., 2016) and PEDv in contaminated animal feed. MCFAs were also shown to prevent transmission of PEDv to 100% of piglets through feed when applied at 10 kg/t

(Dee et al., 2020). However, application of MCFAs at 10 kg/t is not economical.

Although monoglycerides are known to disrupt viral envelopes (Thormar et al., 1987; Yoon et al., 2017), the focus of virus mitigants in feed has largely remained on free fatty acids and the ratios of C6:C8:C10:C12 in free fatty acid blends. As an alternative to free fatty acids and formaldehyde, a proprietary monoglyceride blend was tested in vitro and in a natural feeding behaviour bioassay for efficacy against PEDv in feed. The studies compared the efficacy of the free fatty acid blend to the monoglyceride blend, which was anticipated to inactivate PEDv and prevent disease transmission at a lower inclusion rate. In the bioassay, the proprietary monoglyceride blend performed as well as a highly pure C8 and C10 MCFA blend (1%) and a formaldehyde-based commercial product (Sal CURB®, Kemin Industries, Inc., Des Moines, IA) (3.1 kg/t) when applied to feed at 1.5 kg/t, preventing 100% disease transmission from feed.

2 | MATERIALS AND METHODS

2.1 | Animal care and use

Pigs used in the study were housed in the Pipestone Applied Research biosafety level 2 facility in accordance with the institutional animal care and use guidelines approved by the investigators' ethical review board (Pipestone Applied Research) project number 2021–8.

2.2 | Feed, feed additives and virus application for in vitro virus inactivation

A proprietary monoglyceride blend, a highly pure propriety blend of MCFAs, and Sal CURB® were procured from Kemin Industries, Inc. (Des Moines, IA). Soybean meal-based swine grow/finish feed in mash form was obtained from the feed mill at Southern Research and Outreach Center of the University of Minnesota (Waseca, MN) and confirmed negative for PEDv by real-time RT-PCR. Feed (1 g) was weighed into round-bottom Falcon tubes (12 × 75 mm). The mitigants were diluted 1:100 in sterile distilled water, added to the feed and mixed thoroughly by flicking or shaking the tube 20 times. Each tube, except the negative control, was inoculated with 200 μl PEDv ($\sim 1 \times 10^{5.1}$ $TCID_{50}$/sample). The inoculated feed samples were held at room temperature for 0, 12, 24, 48 and 120 h post-inoculation. At each time point, 2 ml of eluent solution (3% beef extract in 0.05 M glycine) was added, and the sample was vortexed. The feed samples were centrifuged at 1000 RPM for 5 min and the supernatant was transferred to sterile tubes and frozen at −80 °C until the experiment was completed. Each time point for each treatment was performed in triplicate in two independent experiments.

2.3 | In vitro virus recovery

The PEDv was propagated and titrated in Vero-81 cells using Eagle's minimum essential media (MEM) with antibiotics and fetal bovine serum. For virus titration, serial 10-fold dilutions were prepared from thawed eluates followed by inoculation onto monolayers of Vero-81 cells seeded in 96-well, flat bottom plates using 3 wells/dilution. The plates were incubated at 37 °C for 90 min. Unabsorbed virus was decanted and the cells were washed once with Hanks' balanced salt solution. Fresh medium (100 µl, Eagle's MEM supplemented with antibiotics, 10 µg/ml trypsin and 2% FBS) was added to each well and cells were incubated at 37 °C for 5–7 days in a 5% CO_2 atmosphere. Cells were examined daily for the appearance of cytopathic effects (CPE). Virus titres were calculated as $TCID_{50}$ using the Spearman–Karber method (Karber, 1931).

2.4 | Statistical analysis

Data were graphically represented using GraphPad Prism version 9.0.1 for Windows, GraphPad Software, La Jolla, CA. Statistical analysis was conducted using JMP Version 15.0.0, SAS Institute Inc., Cary, NC.

2.5 | Bioassay treatments and feed samples

The experiment consisted of five rooms with six pens in each room. The highly pure MCFAs and the proprietary monoglyceride blend were procured from Kemin Industries, Inc. (Des Moines, IA) and mixed into complete feed at Chandler Feed (Leota, MN). Material application at the mill was conducted the day before pig placement by personnel from Kemin using a Kemin Application Solutions system comprised of a Tuthill 2.3 gear pump and a Horner Programmable Logic Controller. Liquid materials were applied at 120 gal/h through three hydraulic nozzles specified for 0.8 gal/min with a 65° fan pattern positioned in a line above a 3-ton mixer. One sample of each batch of treated feed was collected by a vertical probe of the compartment of the transport truck from the top. The feed samples were analyzed for fatty acid ethyl esters by the customer laboratory service department at Kemin Industries, Inc (Des Moines, IA). From each feed sample, a 5 g subsample was extracted using 25 ml ethanoic sulfuric acid containing nonanoic acid as an internal standard. The feed samples were incubated in the acidified ethanol internal standard solution for 2 h in an 80 °C water bath with shaking at 180 RPM then rested at room temperature to allow the solids to settle. The supernatant liquid (100 µl) was transferred to a new tube containing 2 ml heptane, vortexed, and separated from the aqueous phase using 4 ml of a 10% sodium carbonate in water solution. The top layer containing the fatty acid ethyl esters in heptane was analyzed by gas chromatography-flame ionization detection (GC-FID) using a Restek Rxi-ms column.

2.6 | Site description and housing

The challenge study was conducted at Pipestone's Applied Research BSL-2 facility (Pipestone Veterinary Services, Pipestone, MN) and overseen by Dr. Scott Dee. The PEDv challenge was provided through feed under controlled field conditions and natural feeding behaviour (no gavage). Pigs were acclimated to the environment and the feed for 5 days prior to being exposed to PEDv (pre-challenge period) and then exposed to feed containing PEDv for 20 days (post-challenge period). Feed was provided *ad libitum* for the duration of the trial.

2.7 | Challenge model

Feed for all treatment groups was contaminated using an ice block (454 g in weight/454 ml) containing 100 ml of PEDv Colorado-13 strain at a concentration of 10^5 $TCID_{50}$/ml (Dee et al., 2014). Virus was sourced from the laboratory of Dr. Eric Nelson at South Dakota State University (SDSU). The balance of the liquid for the block consisted of MEM. On day 0 and 6 of the post-challenge period, one block was dropped into each of the designated feed bins through the opening at the top of the bin. The ice block was carried by gravity and immersed into the feed where it melted and permeated the feed. The feed was augured into the animal rooms over time and pigs consumed it using natural feeding behaviour.

2.8 | Pigs

Nursery pigs between 42 and 49 days of age were sourced from Pipestone and tested negative for porcine reproductive and respiratory virus (PRRSV), PEDv and Senecavirus A (SVA). The pigs were weighed and distributed equally by weight across 6 pens in 5 rooms (10 or 11 pigs/pen; total 323).

2.9 | Monitoring and sampling

Environmental samples were collected from each pen (6 per room) on trial days 6 and 15. Environmental samples included both oral fluids collected by hanging rope and pen-based feed samples collected using dry Swiffer sweeping cloths to swab inside the feeder bin. After swabbing with the dry Swiffer, the cloths were immersed in 20 ml of phosphate buffered saline (PBS) and wrung into disposable plastic bags, one per sample. A 3 ml aliquot of the Swiffer PBS sample was decanted into individual snap cap Falcon tubes and frozen at −80 °C until testing. Rectal swabs were collected on day 20 from each pig for PEDv diagnosis. Also on day 20, the presence of diarrhea was determined as a "yes/no" for each pig based on visual soiling of the perineal area and the consistency of the stool observed during swabbing. All samples, rectal swabs, oral fluids, and Swiffer PBS, were analysed by qPCR at the South Dakota State University Veterinary Diagnostic Laboratory (SDSU VDL, Brookings, South Dakota), and determined to be positive or negative by the Ct (cycle time) value. Samples with a cycle number greater than 40 were determined negative for PEDv. Pigs were weighed individually on trial days −5 and 20 and the average daily gain (ADG) performance statistics were calculated for each pen after adjusting for mortality. The

TABLE 1 Inclusion rates of mitigants in feed to determine in vitro inactivation of PEDv

Treatment	Inclusion rate (kg/t)	Treatment (µl)	Virus (µl)
Positive control	0	None	200
MCFA	10	1000	200
Sal CURB	3.1	310	200
Monoglyceride	1.5	150	200
Monoglyceride	2.5	250	200
Monoglyceride	3.5	350	200
Negative control	0	None	None

Note: All mitigants were diluted 1:100 with sterile water, added to 1 g of feed contained in Falcon tubes and mixed by hand shaking. The PEDv was added approximately 10 min after the feed was treated with mitigants.
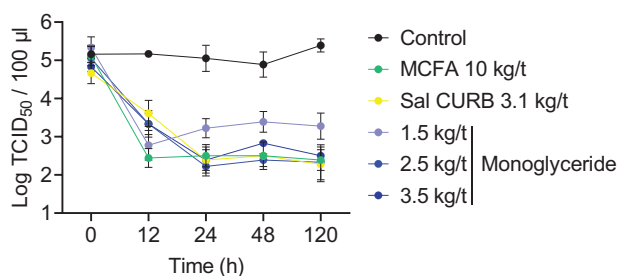
FIGURE 1 Viability of PEDv over time in feed treated with mitigants. Symbols represent the mean $\pm$ SD, $n = 6$ from 2 independent experiments

individual pig was the experimetnal unit for PEDv diagnosis, and the pen was the experimental unit for performance.

3 | RESULTS

3.1 | Inactivation of PEDv in vitro

The results of in vitro experiments shown in Tables 1 and 2 and Figure 1 indicate that MCFA at 10 kg/t was able to inactivate 99.79% of the virus within 12 h of contact. It further indicates that the extent of virus inactivation by MCFA at 10 kg/t did not increase appreciably at 24, 48 and 120 h of contact. It took 24 h for Sal CURB at 3.1 kg/t to inactivate approximately 2 logs (99%) of the virus. When used at 1.5 kg/t, the proprietary monoglyceride blend was able to inactivate 2 logs (99%) of the virus within 24 h. The difference in virus inactivation by 2.5 or 3.5 kg/t of monoglyceride was not significant. At 2.5 kg/t, the proprietary monoglyceride blend inactivated 2 logs of virus within 12 h of contact.

3.2 | Liquid product application

The highly pure MCFA and the proprietary monoglyceride blends were applied to independently mixed, 2-standard ton batches in a 3-

standard ton mixer and mixed for 180 s after full application of the liquids. The feed was mixed in the following order to reduce contamination across batches: untreated control, proprietary monoglyceride blend at 1.5 kg/t, 2.5 kg/t, then 3.5 kg/t and the highly pure MCFA blend at 10 kg/t was applied last (Table 3). On day 6 post-challenge, the feeder system for treatment 4 (2.5 kg/t monoglyceride) malfunctioned and a large amount of feed was lost in the manure pit under one of the 6 pens. A second, 2-standard ton batch of feed was mixed and the monoglyceride was applied at 2.5 kg/t to replace the lost feed. Feed samples were analysed for fatty acid ethyl esters and the results indicate that both the highly pure MCFA and the proprietary monoglyceride blends were applied correctly (Table 3).

3.3 | PCR results of environmental swabs (feeder) and oral fluids (ropes)

The presence of PEDv RNA was detected by PCR in feeder and oral fluid samples from all rooms on day 6 and 15 post-challenge. All six environmental swab samples collected from the feeders and all six oral fluid samples in the untreated control group were positive for PEDv indicating that PEDv was likely delivered into the experimental rooms via the feed (Table 4). The MCFA positive control treatment effectively reduced the positivity rate in all environmental samples by 50% (Table 4). The proprietary monoglyceride blend treatment level correlated with reduced PEDv positivity in the environmental swabs on day 6 and 15 post-challenge. Specifically, on day 15 the number of positive oral fluid samples were 2, 3 and 4 out of a total of 6 for the 3.5, 2.5 and 1.5 kg/t monoglyceride treatments, respectively (Table 4). These data suggest a dose-dependent effect of the monoglyceride on virus stability in feed. Although a positive PCR result from the environmental samples indicates that virus was present and does not effectively determine the viability of the virus, the data suggest that viral RNA was degraded in the feed of the MCFA- and proprietary monoglyceride blend-treated groups.

3.4 | Clinical diarrhoea scores and PEDv diagnostics

The presence or absence of diarrhoea for each pig was recorded on day 20 post-challenge. No clinical evidence of diarrhoea was noted in rooms 1–3, whereas 4 pigs showed evidence of diarrhoea in room 4 (Table 5). In contrast, diarrhoea was observed in 21/62 pigs in the untreated control room. To determine if the diarrhoea is related to PEDv infection, all pigs were swabbed rectally and tested for PEDv by PCR at the end of the 20-day challenge period. In the untreated control room 34/62 pigs tested positive for PEDv, which is a higher diagnostic incidence of PEDv than was observed by the clinical diarrhoea scoring (Table 5). Conversely, rectal swabs were negative for PEDv in all the pigs from the treated groups including the lowest inclusion rate, 1.5 kg/t monoglyceride, group (Table 5). The diagnostic data indicates that the monoglyceride at the lowest tested inclusion rate of 1.5 kg/t

TABLE 2 Inactivation of PEDv by mitigants at different time points

Treatment (application)	Log TCID$_{50}$ /100 µl of PEDv at indicated time				
	0 h	12 h	24 h	48 h	120 h
Positive control	5.16[ab]	5.17[a]	5.05[a]	4.89[a]	5.39[a]
MCFA (10 kg/t)	5.11[ab]	2.45[c]	2.50[c]	2.50 cd	2.39[c]
Sal CURB (3.1 kg/t)	4.83[c]	3.61[b]	2.39[c]	2.50 cd	2.28[c]
Monoglyceride (1.5 kg/t)	5.33[a]	3.78[c]	3.22[b]	3.39[b]	3.28[b]
Monoglyceride (2.5 kg/t)	5.05[abc]	3.33[b]	2.22[c]	2.39[d]	2.33[c]
Monoglyceride (3.5 kg/t)	4.83[bc]	3.33[b]	2.38[c]	2.83[c]	2.50[c]

Note: Values in the same column that are not connected by the same superscript letter are significantly different, $p < .05$. One-way ANOVA followed by Tukey–Kramer test for all pairs, $n = 6$ from two independent experiments.
Abbreviation: MCFA, medium chain fatty acids.

TABLE 3 Measured application rate of mitigants on feed

Treatment	Order of application	Target inclusion rate (kg/ton)	Product recovery from feed (kg/t)
Untreated control	1	0	0
MCFA	5	10	10.5
Monoglyceride	2	1.5	1.4
Monoglyceride	3	2.5	2.4
Monoglyceride	4	3.5	3.6
Monoglyceride repeat	NA	2.5	3.0

Note: Samples of feed were analysed for total C8 and C10 fatty acids by gas chromatography.
Abbreviations: MCFA, medium chain fatty acid blend; NA, not applicable.

TABLE 4 Detection of PEDv in the environment

Treatment (% inclusion)	Feeders (+/total)		Oral fluids (+/total)	
	Day 6	Day 15	Day 6	Day 15
Untreated control	6/6	6/6	6/6	6/6
MCFA (10 kg/t)	2/6	2/6	1/6	3/6
Monoglyceride (1.5 kg/t)	2/6	4/6	1/6	5/6
Monoglyceride (2.5 kg/t)	0/6	3/6	0/6	2/6
Monoglyceride (3.5 kg/t)	1/6	2/6	1/6	2/6

Note: Environmental swabs from the pen feeder and oral fluids were analysed for PEDv by PCR on day 6 and 15 post-challenge.

was 100% effective in preventing transmission of PEDv over 20 days from contaminated feed.

3.5 | *E. coli* F18 outbreak

On day 4 post-challenge, the site manager reported signs of elevated mortality with cyanosis of extremities in rooms 1–3. Necropsy indicated lesions of haemorrhagic enteritis with gas in multiple pigs, suggestive of *E. coli* septicaemia (Figure 2). All animals in the study were

TABLE 5 Incidence of PEDv transmission to pigs from feed

Treatment (% inclusion)	PEDv+ pigs[†] (+/total)	Diarrhoea incidence[‡] (+/total)	Room no.
Untreated control	34/62	21/62	5
MCFA (10 kg/t)	0/53	0/53	1
Monoglyceride (1.5 kg/t)	0/64	4/64	4
Monoglyceride (2.5 kg/t)	0/48	0/48	3
Monoglyceride (3.5 kg/t)	0/57	0/57	2

Note: All treatment groups were challenged via feed with an ice block containing 5 logs TCID$_{50}$ PEDv placed in the feeder bin.
[†] PEDv was diagnosed via rectal swabs collected on day 20 post-challenge from all surviving pigs and analysed at the South Dakota State University veterinary diagnostic laboratory.
[‡] Diarrhoea incidence was determined by facility manger on day 20 post-challenge.
Abbreviation: MCFA, medium chain fatty acids (free fatty acids).

FIGURE 2 Septicaemia caused by *Escherichia coli*. Lesions of pig infected with *E. coli* F18. Note reddened, gaseous intestinal tract

immediately treated intramuscularly with enrofloxacin (Baytril, Elanco, Greenfield, IN) and orally via the drinking water with gentamycin. *E. coli* positive for F18 pillus and LT, STa, STb, Stx2 and Stx2e toxins were recovered from two sets of tissue samples sent to Iowa State

TABLE 6 Performance and mortality over 20-day challenge period

Treatment	Starting inventory	Mortalities	Final inventory	ADG (lb)[1]	Room
Untreated control	65	3	62	1.00 ± 0.38^a	5
MCFA (10 kg/t)	65	12	53	0.81 ± 0.25^a	1
Monoglyceride (1.5 kg/t)	65	1	64	1.20 ± 0.18^a	4
Monoglyceride (2.5 kg/t)	64	16	48	0.75 ± 0.13^a	3
Monoglyceride (3.5 kg/t)	64	7	57	0.91 ± 0.19^a	2

Note: Rooms 1–4 were negatively impacted by *E. coli* F18 outbreak. All pigs in all rooms were treated with antibiotics intramuscularly by injection and orally through drinking water equally, regardless of *E. coli* severity.

[1]ADG (average daily gain) was adjusted for mortality and is shown as the mean $\pm$ SD. Values in the same column that are not connected by the same superscript letter are significantly different, $p < .05$. One-way ANOVA followed by Tukey–Kramer test for all pairs, $n = 6$.

University. No evidence of PEDv was observed in the tissues. Animals exhibited reduced growth and elevated mortality (Table 6).

4 | DISCUSSION

Biosecurity is an essential element of modern pig production, which has progressively been trending towards larger farms and more pig movement across regions since the 1980s. Diseases introduced from outside the facility by people, vehicles, new animals, pests and feed can seriously impact efficiency. One piece of the biosecurity puzzle is feed. The efficacy of MCFAs against swine viruses in feed is a promising start, yet there is still a need for a more effective, less expensive and safe virus mitigants.

The efficacy of a proprietary blend of monoglycerides at reducing PEDv viability in feed was evaluated to find solutions to improve feed biosecurity. In vitro data concluded that the monoglyceride effectively reduced PEDv viability in feed from 5 logs $TCID_{50}$ to < 3 logs $TCID_{50}$ when included at 2.5 and 3.5 kg/t. The 1.5 kg/t monoglyceride treatment reduced $TCID_{50}$ of PEDv by approximately 1.5 log in the in vitro feed assay. In the PEDv ice-block challenge model, which delivered the virus through natural feeding behaviour for 20 days, the monoglyceride at the lowest tested inclusion rate of 1.5 kg/t prevented 100% of disease transmission to piglets. The efficacy demonstrated in the ice-block challenge model suggests that monoglycerides are just as effective as MCFAs yet can be added to feed at a lower inclusion rate than MCFAs to inactivate PEDv.

The research approach was focused on comparing the efficacy of the proprietary monoglyceride blend with the known efficacy of the high purity MCFA blend against PEDv. However, other enveloped viruses and bacterial pathogens were not evaluated. Thus, the demonstrated efficacy of the monoglyceride is limited to one, enveloped virus pathogen, whereas the efficacy of MCFAs has been demonstrated against *Salmonella*, SVA and PRRSv in the ice-block challenge model. Furthermore, MCFAs and glycerol monolaurate have been shown to be effective against the capsid-protected DNA African swine fever virus (ASFv) in vitro (Jackman et al., 2020). More efficacy information needs to be collected on the proprietary monoglyceride blend evaluated in this study before the material can be considered equally or more effective than the free medium chain fatty

acids. Additionally, the lowest tested monoglyceride inclusion rate of 1.5 kg/t prevented disease transmission, so the minimum effective application rate remains unknown. Additional experiments conducted using the monoglyceride at <1.5 kg/t could reveal the point at which the material no longer provides effective protection in feed.

During the study, the outbreak of F18 pathogenic *E. coli* impacted animal performance (ADG) and the quality of the study. Because the outbreak disproportionately affected the experimental treatment groups (Table 6), the performance benefits from preventing PEDv infection compared to the untreated group were obscured. The *E. coli* diagnosis was accompanied by visual inspection of the affected pigs, which exhibited gaseous, reddened intestines (Figure 2). To determine PEDv infection, only the rectal swab was used for diagnosis on day 20. The pigs in the trial were sourced from a PEDv-negative herd that was monitored monthly using serology and oral fluids. However, at the conclusion of the trial, the pigs were not checked for antibodies to PEDv. And with the *E. coli* outbreak causing clinical symptoms and distress, a clinical PEDv diagnosis could have been missed. Future studies could incorporate additional timepoints for diagnostic testing by rectal swab, a serological survey of PEDv antibodies in the blood, and histopathology of the intestine to ensure the feed mitigants prevent infection rather than dampen the infection.

This discovery of the proprietary monoglyceride blend's efficacy against PEDv in feed brings into focus a new group of molecules. Monoglycerides of all lengths and compositions should be studied for efficacy against various pathogens in feed. The continued threat of feed-resident pathogens drives research for alternatives to antibiotics and formaldehyde-based products, yet there are still few cost-effective options available. Monoglycerides further expand the possibilities for solutions to a growing problem. Intensification of pig population densities as well as personnel and facility biosecurity could be undermined by insufficient feed biosecurity practices. The use of additives and handling and transport protocols to improve feed quality and safety are needed to ensure optimal pig performance and secure local and global food supply chains.

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST

Kemin Industries sponsored this research and is the supplier of the proprietary monoglyceride and medium-chain fatty acid blends. FP, JR and MP are employees of Kemin Industries, Inc.

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