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K-STATE Research and Extension

2021 ANIMAL FEED AND PET FOOD RESEARCH

Feed Mitigant Efficacy for Control of Porcine Epidemic Diarrhea Virus and Porcine Reproductive and Respiratory Syndrome Virus when Inoculated Alone or Together in Feed

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Summary

Research has demonstrated that swine feed can be a fomite for viral transmission and feed additives can reduce viral contamination. Therefore, the objective of this study was to evaluate two feed additives in feed contaminated with PEDV or PRRSV. Feed additives included: no treatment, 0.33% commercial formaldehyde-based product, and 0.50% medium chain fatty acids (MCFA) blend. Feed samples were inoculated with PEDV and PRRSV alone or together at an inoculation concentration of 10^6 $TCID_{50}$/g for each virus. Once inoculated, feed was stored at room temperature for 24 h before analyzing via qRT-PCR. For samples inoculated with PEDV or PRRSV alone, a quantitative real time reverse transcription PCR (qRT-PCR) assay was used, which was designed to detect PEDV or PRRSV nucleic acid. For co-inoculated samples, an assay was designed to detect PEDV and PRRSV within a single assay. For PEDV alone, there was marginally significant evidence that feed additives resulted in differences in cycle threshold (Ct) value ($P = 0.052$), but no evidence was observed for pairwise differences. For PRRSV alone, formaldehyde increased Ct compared to the untreated control and MCFA treatment ($P < 0.05$). For co-infection of PRRSV and PEDV, MCFA and formaldehyde increased Ct ($P < 0.05$) in comparison to non-treated feed. In summary, formaldehyde increased Ct values in feed when contaminated with PRRSV while both feed additives increased Ct in feed when co-inoculated with PRRSV and PEDV. This study also provided evidence that the co-inoculation model can effectively evaluate mitigants.

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Introduction

The suspected mode of entry for PEDV into the United States during the 2013 and 2014 outbreak was in contaminated feed ingredients.[4] Consequently, this taught us that feed biosecurity should be enhanced as part of standard farm biosecurity practices. While most of the work regarding feed as a potential vector for viral disease transmission has focused on PEDV, research has demonstrated that other viruses such as PRRSV, foot and mouth disease, and other foreign animal diseases could survive in feed under transatlantic shipping conditions.[5] These findings highlight that while foreign trade can potentially transmit novel viruses across geographical borders, these findings also stress the importance of applying biosecurity to the feed supply chain to control the spread of endemic viruses like PRRSV and PEDV. In general, if contaminated feed is a concern for any swine production system, there are multiple solutions to this problem but the most common is use of feed additives to reduce virus viability. These feed additives have been shown to be successful with mitigating risk in feed experimentally inoculated with PEDV,[6] but there is a lack of data concerning feed mitigant efficacy in the presence of more than one virus in feed. Therefore, the objective of this study was to evaluate the efficacy of two feed additives to increase Ct values of feed when inoculated with PEDV or PRRSV or inoculated with both of these viruses.

Materials and Methods

General

A corn and soybean meal-based swine gestation diet was used. No evidence for contamination was observed in the test diet for PEDV and PRRSV using quantitative real time reverse transcription PCR (qRT-PCR) prior to the study. The treatments for this study included feed inoculated with PEDV alone, PRRSV alone, or co-inoculated with both viruses; and feed additives included no treatment, 0.33% commercial formaldehyde-based product (Sal Curb, Kemin Industries, Des Moines, IA), or 0.50% MCFA blend (MCFA; 1:1:1 ratio of C6:C8:C10, Sigma Aldrich, St. Louis, MO). Chemical treatments were added to 100-g batches of feed and mixed for 15 min in a mason jar mixer (Central Machine Shop, Purdue University, West Lafayette, IN). For treatments consisting of a single virus inoculate, 22.5 g of treated feed was placed into three separate polyethylene bottles (250 mL Nalgene bottle, square wide-mouth high-density polyethylene; Thermo Fisher Scientific, Waltham, MA). For treatments containing the co-inoculate, 20 g of feed was placed into three separate 250 mL polyethylene bottles to ensure equal virus titer levels for the individual virus across all treatments. All treatments had three replicates for this study. Polyethylene bottles were stored at room temperature for 24 h before inoculation.

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[4] USDA-APHIS. United States Department of Agriculture Animal and Plant Health Inspection Service Veterinary Services (2015). Swine enteric coronavirus introduction to the United States: Root cause investigation report. Accessed February 24, 2021.

[5] Dee, S. A., Bauermann, F. V., Niederwerder, M. C., Singrey, A., Clement, T., de Lima, M., ... & Petrovan, V. (2018). Survival of viral pathogens in animal feed ingredients under transboundary shipping models. *PloS ONE*, *13*(3). doi:10.1371/journal.pone.0194509.

[6] Gebhardt, J.T., J.C. Woodworth, M.D. Tokach, J.M. DeRouchey, R.D. Goodband, C.K. Jones, and S.S. Dritz. 2020. Effect of dietary medium-chain fatty acids on nursery pig growth performance, fecal microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage. J. Anim. Sci. 98(1):1-11. doi:10.1093/jas/skz358.

Inoculation

The samples were inoculated at the Kansas State University Veterinary Diagnostic Laboratory (KSVDL), Manhattan, KS, with PEDV, PRRSV, or both viruses with an initial concentration of 10^7 $TCID_{50}$/mL. All treatments were inoculated by pipetting 2.5 mL of each viral inoculum into each bottle as required for the treatment to result in a final viral concentration of 10^6 $TCID_{50}$/g of feed. Bottles were then shaken for 15 s to distribute the virus through the feed.

Laboratory analysis

Bottles were held at room temperature for 24 h after inoculation. Then, 100 mL of phosphate buffered saline (PBS) was added to each inoculated bottle and shaken to ensure even mixing. Bottles were placed in a refrigerator at 39°F for 24 h to allow feed to settle. Supernatant was collected and placed into a 96 well plate for qRT-PCR. Quantitative real time reverse transcription PCR was conducted using methods previously described by Gebhardt et al.[7] Briefly, supernatant was extracted using a Kingfisher 96 magnetic particle processor (Fisher Scientific, Pittsburgh, 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. Samples inoculated with PEDV or PRRSV alone were analyzed using a qRT-PCR assay designed for PEDV or PRRSV nucleic acid. For co-inoculated samples, an assay was designed by the KSVDL to independently detect both PEDV and PRRSV nucleic acid within a single reaction. All responses from the qRT-PCR reactions were reported in Ct value.

Statistical analysis

Statistical analysis for this study was performed using the PROC GLIMMIX procedure in SAS (v. 9.4, SAS Institute, Inc., Cary, NC). This study was a completely randomized design with the effect of mitigant type (none, formaldehyde, and MCFA) analyzed for each inoculation type (PEDV alone, PRRSV alone, or both viruses). The experimental unit was each 250 mL polyethylene container. If inoculated samples had no detectable RNA, a value of 45.0 was assigned to the sample for statistical analysis. A Tukey multiple comparison adjustment was incorporated when appropriate. Results were considered significant at $P \leq 0.05$ and marginally significant at $P \leq 0.10$.

Results and Discussion

For PEDV alone, there was marginally significant evidence that feed additives resulted in differences in Ct value ($P = 0.052$; Table 1), but no evidence was observed for pairwise differences. For PRRSV alone, formaldehyde increased Ct compared to the untreated control and MCFA treatment ($P < 0.05$). For co-infection of PRRSV and PEDV, MCFA and formaldehyde increased Ct ($P < 0.05$) in comparison to non-treated feed.

[7] Gebhardt, J. T., J. C. Woodworth, C. K. Jones, M. D. Tokach, P. C. Gauger, R. G. Main, J. Zhang, Q. Chen, J. M. DeRouchey, R. D. Goodband, C. R. Stark, J. R. Bergstrom, J. Bai, and S. S. Dritz. 2018. Determining the impact of commercial feed additives as potential porcine epidemic diarrhea virus mitigation strategies as determined by polymerase chain reaction analysis and bioassay. Transl. Anim. Sci. 3:93-102. doi: 10.1093/tas/txy100.

In summary, formaldehyde effectively increased Ct values in feed when contaminated with PRRSV alone, while both mitigants effectively increased Ct values in feed when co-inoculated with PRRSV and PEDV. Both mitigants were marginally effective in feed when contaminated with PEDV alone. This study also supplied evidence that the co-inoculation model can effectively evaluate mitigants.

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Table 1. Cycle threshold values for PEDV and PRRSV when inoculated either alone or together following application of feed additives[1]

		Mitigant				
Virus	**Assay**	**Untreated**	**MCFA[2]**	**Formaldehyde[3]**	**SEM**	***P* =**
PEDV	PEDV	31.2	33.5	34.2	0.69	0.052
PRRSV	PRRSV	30.0[b]	34.2[b]	42.0[a]	1.78	0.009
Co-inoculated						
PEDV quantification	Duplex	30.8[b]	31.9[a]	32.5[a]	0.23	0.006
PRRSV quantification	Duplex	30.0[b]	33.8[a]	34.7[a]	0.85	0.019

[1] Swine feed samples were inoculated with 10^6 $TCID_{50}$/g of PEDV, PRRSV, or PEDV and PRRSV co-inoculation then analyzed using one of three quantitative real time reverse transcription polymerase chain reaction assays, including an assay detecting PEDV only, an assay detecting PRRSV only, or an assay detecting and independently reporting quantification of both PEDV and PRRSV (duplex).
[2] Medium chain fatty acid blend (MCFA; 1:1:1 ratio of C6:C8:C10; Sigma Aldrich, St. Louis, MO) at 0.50% inclusion.
[3] Commercial formaldehyde-based feed additive (SalCURB; Kemin Industries, Des Moines, IA) at 0.33% inclusion.
[a,b,c] Means within row lacking common superscripts differ, $P < 0.05$.