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Efficacy of Feed Additives Against Swine Viruses in Feed

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Summary

Research has demonstrated that swine viruses can be transmitted via feed. Therefore, strategies are needed to prevent or mitigate swine viruses in feed. The use of chemical feed additives is a strategy that has been shown to have potential utility for this purpose. Therefore, the objective of this study was to evaluate the efficacy of a commercially available formaldehyde-based feed additive, medium chain fatty acid blend (MCFA), and commercially available fatty acid-based products for mitigation of porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome virus (PRRSV) as viral mitigants in a feed matrix. Experimental treatments consisted of: 1) non-treated, individually inoculated virus controls (positive control); 2) 0.33% commercial formaldehyde-based product (Sal Curb; Kemin Industries, Inc.; Des Moines, IA); 3) 0.50% MCFA blend (1:1:1 ratio of C6:0, C8:0, and C10:0, Sigma Aldrich, St. Louis, MO); 4) 0.25%; 5) 0.50%; or 6) 1.00% of commercial dry mono and diglyceride-based product (Furst Strike; Furst-McNess Company, Freeport, IL); 7) 0.25%; 8) 0.50%; or 9) 1.00% of commercial dry mono and diglyceride-based product (Furst Protect; Furst-McNess Company, Freeport, IL); 10) 0.25%; 11) 0.50%; or 12) 1.00% dry mono and diglyceride-based experimental product (Furst-McNess Company, Freeport, IL). In total there were 12 treatments with 3 replications per treatment. A complete swine feed was treated with each chemical treatment before inoculation with 10^6 $TCID_{50}$/g of feed with PEDV or PRRSV. Post-inoculation feed was held at ambient temperature for 24 h before being analyzed via quantitative real time reverse transcription PCR (qRT-PCR). The analyzed values represent the cycle threshold (Ct). A lower Ct value indicates a higher level of detectable viral nucleic acid. Formaldehyde and MCFA decreased ($P < 0.05$) the detectable RNA concentration of PEDV and PRRSV compared to all other treatments. Furst Strike, Furst Protect, and the experimental product did not significantly reduce detectable concentrations of RNA for

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PEDV or PRRSV. In conclusion, MCFA and formaldehyde chemical treatments are effective at reducing nucleic acid levels of PEDV and PRRSV in feed.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) was first seen in the United States in the late 1980s. The virus causes reproductive distress including early farrowing and late term abortions, along with high pre-wean mortality weights. In older pigs it can cause respiratory distress including pneumonia, dysphonia, fever, and stunting due to disease.[6] Porcine epidemic diarrhea virus (PEDV) was introduced into the United States in 2013 and became prevalent throughout 2015. The virus causes 90–100% mortality in pre-weaned piglets and can cause reductions in growth in older pigs.[7] Research has demonstrated that viruses can be transmitted via swine feed.[8,9] The contamination of feed can be caused by contaminated ingredients, transport, previously contaminated manufacturing surfaces, and during feed storage through dust and fecal matter. Feed mills can become contaminated with viruses via contaminated feed ingredients, worker and visitor foot traffic, and receiving or delivery trucks.[10,11] This information has generated interest in determining the effects of different chemical mitigants for feed and commercial feed additives on viruses in feed. Previous research has evaluated the effects of medium chain fatty acids (MCFA), essential oils, organic acids, and formaldehyde.[12] Research has shown the efficacy of 0.5–1.0% inclusion of a 1:1:1 ratio blend of MCFA (hexanoic, C6:0; octanoic, C8:0; and decanoic, and C10:0 acids) and the inclusion of 0.325% formaldehyde significantly reduced PEDV RNA levels in swine feed.[7,13] All of the previous research has been focused on PEDV and no research has examined the effect of formaldehyde or MCFA against PRRSV. Along with testing

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[7] The Pig Site. 2020. Porcine epidemic diarrhea (PED). Available from: https://thepigsite.com/disease-guide/porcine-epidemic-diarrhoea-ped-scour.

[8] Cochrane, R.A, S.S. Dritz, J.C. Woodworth, C.R. Stark, M. Saensukjaroenphon, J.T. Gebhardt, J. Bai, R.A Hesse, E.G. Poulsen, Q. Chen, P.C. Gauger, R.J. Derscheid, J. Zhang, M.D. Tokach, R.G. Main, C.K. Jones. 2020. Assessing the effects of medium-chain fatty acids and fat sources on PEDV infectivity, T. Anim. Sci, 4(2):1051–1059. doi:10.1093/tas/txz179.

[9] Lerner, A.B., R.A. Cochrane, J.T. Gebhardt, S.S. Dritz, C.K. Jones, J.M. DeRouchey, M.D. Tokach, R.D. Goodband, J. Bai, E. Porter, J. Anderson, P.C. Gauger, D.R. Magstadt, J. Zhang, B. Bass, T. Karnezos, B. de Rodas, J.C. Woodworth. 2020, Effects of medium chain fatty acids as a mitigation or prevention strategy against porcine epidemic diarrhea virus in swine feed. J. Anim. Sci. 98(6):1-6. doi:10.1093/jas/skaa159.

[10] Cochrane, R.A., S.S. Dritz, J.C. Woodworth, C.R. Stark, A.R. Huss, J.P. Cano, R.W. Thompson, A.C. Fahrenholz, and C.K. Jones. 2016. Feed mill biosecurity plans: a systematic approach to prevent biological pathogens in swine feed. J. Swine Health Prod. 24(3):154–164.

[11] Schumacher, L.L., A.R. Huss, R.A. Cochrane, C.R. Stark, J.C. Woodworth, J. Bai, E.G. Poulsen, Q. Chen, R.G. Main, J. Zhang, P.C. Gauger, A. Ramirez, R.J. Derscheid, D.M. Magstadt, S.S. Dritz, and C.K. Jones. 2017. Characterizing the rapid spread of porcine epidemic diarrhea virus (PEDV) through an animal food manufacturing facility. PLoS ONE. 12:e0187309. doi:10.1371/journal.pone.0187309.

[12] 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, S.S. Dritz. 2019. Determining the impact of commercial feed additives as potential porcine epidemic diarrhea virus mitigation strategies as determined by polymerase chain reaction analysis and bioassay. T. Anim. Sci. 3(1):93–102. doi:10.1093/tas/txy100.

[13] 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

these proven products, questions arise about other products and fatty acids and their efficacy as viral mitigants in feed. For example, Furst Protect was demonstrated to be effective in protecting pigs from feed contaminated with PRRS, PEDv and SVA in an in vivo model.[14] Therefore, the objective of this study was to evaluate the efficacy of commercial formaldehyde, MCFA, and commercially available fatty acid-based products against PEDV and PRRSV as viral mitigants in a feed matrix.

Procedures

A complete corn and soybean meal-based swine gestation diet (Table 1) was utilized—it did not contain specialty ingredients (whey, specialty soybean meal, animal plasma protein, or fish products) or antibiotics. All feed samples tested negative for PEDV and PRRSV by quantitative real time reverse transcription PCR (qRT-PCR) prior to chemical treatment. Eleven chemical treatments were applied to the diet for each virus separately. Treatments included in the experiment were: 1) negative control with no chemical addition; 2) 0.33% of the liquid commercial formaldehyde-based product (Sal Curb, Kemin Industries, Des Moines IA); 3) 0.50% of a liquid MCFA blend (1:1:1 ratio of C6:0, C8:0, and C10:0, Sigma Aldrich, St. Louis, MO); 4) 0.25%; 5) 0.50%; or 6) 1.00% of a dry commercial mono and diglyceride-based product (Furst Strike, Furst-McNess Company, Freeport, IL); 7) 0.25%; 8) 0.50%; or 9) 1.00% of a dry commercial mono and diglyceride-based product (Furst Protect, Furst-McNess Company, Freeport, IL); 10) 0.25%; 11) 0.50%; or 12) 1.00% of a prototype dry mono and diglyceride-based experimental product. In total 12 treatments with 3 replications per treatment were made for PEDV and PRRSV individually. Chemical treatments were added to 0.22 lb batches of feed and mixed for 15 minutes in a mason jar mixer (Central Machine Shop, Purdue University, West Lafayette, IN) with 10 5/16 in hex nuts for agitation. Treated feed (0.05 lb) was placed into three separate polyethylene bottles (250 mL Nalgene bottle, square wide-mouth high-density polyethylene; Thermo Fisher Scientific, Waltham, MA) to achieve 3 replicates per treatment. Polyethylene bottles were stored at ambient temperature for 24 h before inoculation.

Inoculation

The samples were inoculated in the polyethylene container at the Kansas State University Veterinary Diagnostic Laboratory with either the PEDV USA/Co/2013 (KF272920.1) or the PRRSV 1-7-4. Both PEDV and PRRSV were provided by the Animal Disease Research and Diagnostic Laboratory at South Dakota State University. Each viral inoculum contained an infectious titer of 10^7 $TCID_{50}$/mL. All treatments were inoculated by pipetting 2.5 mL of each viral inoculum individually into each bottle, resulting in a final viral concentration of 10^6 $TCID_{50}$/g of feed. Bottles were then shaken for 15 s to distribute each virus throughout the feed matrix.

Bottles were held at room temperature for 24 h before 100 mL of phosphate buffered saline (PBS; pH 7.2 1X, Life Technologies, Grand Island, NY) was added to each inoculated bottle and shaken to ensure even mixing. Bottles were then placed in a refrig-

microbial composition, and mitigation properties against porcine epidemic diarrhea virus following storage. J. Anim. Sci. 98(1):1-11. doi:10.1093/jas/skz358.

[14] Dee, S.A., M. C. Niederwerder, R. Edler, D. Hanson, A. Singrey, R. Cochrane, G. Spronk, and E. Nelson. 2020. An evaluation of additives for mitigating the risk of virus-contaminated feed using an ice-block challenge model. Trans. Bound. And Emer. Dis. 00:1-3. doi: 10.1111/tbed.13749.

erator at 39.2°F for 24 h to allow feed to settle. Quantitative real time reverse transcription PCR was conducted for all treatments with each virus tested based upon assays designed for each specific virus' genetic material (singleplex). Supernatant was collected and placed into a 96-well plate for qRT-PCR. Supernatant from the 96-well plate was 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 -122°F until assayed by qRT-PCR. Analyzed values indicate the cycle threshold at which virus was detected. A high level indicates a lower amount of detectable nucleic acid, this however, does not necessarily mean less infectivity.

Data Analysis
Each 250 mL bottle was considered as an experimental unit resulting in 3 replicates per treatment, with fixed effects of chemical treatments and virus inoculum. Data were analyzed using the PROC GLIMMIX procedure in SAS (SAS Institute 9.4, Inc. Cary, NC). Results were considered significant if $P \leq 0.05$ and a marginally significant if $P \leq 0.10$.

Results and Discussion
Formaldehyde and MCFA treatments decreased ($P < 0.05$) detectable PEDV RNA in treated feed compared to the untreated control, Furst Protect, Furst Strike, and Prototype A products. Furst Protect, Furst Strike, and Prototype A did not reduce detectable PEDV RNA in treated feed regardless of treatment level as compared to the untreated control (Table 2).

Formaldehyde addition to feed decreased ($P < 0.05$) PRRSV RNA in inoculated feed as compared to all other treatments. Two of the three formaldehyde samples contained no detectable PRRSV RNA. Adding MCFA to the diet decreased ($P < 0.05$) detectable PRRSV RNA in inoculated feed as compared to the untreated control, Furst Protect, Furst Strike, and Prototype A. Formaldehyde and MCFA had similar results in reducing detectable PEDV RNA. Formaldehyde, however, resulted in a larger reduction ($P < 0.05$) of detectable PRRSV RNA compared to MCFA. Furst Protect, Furst Strike, and Prototype A did not significantly reduce detectable PRRSV RNA in treated feed regardless of product concentration as compared to the untreated control.

In conclusion, Furst Protect, Furst Strike, and Prototype A did not reduce the detectable RNA of either PEDV or PRRSV. Commercial formaldehyde and the MCFA blend both significantly reduced the detectable RNA of either PEDV or PRRSV. Formaldehyde and MCFA products were both liquid, whereas the commercial products were dry products. These data raises the question of whether physical product form influences the mitigant efficacy when studied in an in vitro model. Future research is needed to develop adequate models for the swine gastrointestinal tract to evaluate how mitigant product form (liquid or dry) influences efficacy.

Table 1. Diet composition, as-fed basis

Ingredient	%
Corn	78.40
Soybean meal, 46.5%	17.27
Soy oil	0.50
Monocalcium phosphate 21%	1.30
Limestone	1.30
Salt	0.50
Trace mineral premix[1]	0.15
Vitamin premix[2]	0.25
Sow add pack[3]	0.25
Phytase[4]	0.08
Total	100

[1] Provided 1.36 g Cu from copper sulfate; 72.72 mg Ca from calcium iodate; 14.09 mg Fe from ferrous sulfate; 1.36 g Mn from manganese sulfate; 54.54 mg Se from sodium selenite; and 14.09 g Zn from zinc sulfate per lb of premix.

[2] Provided 750,000 IU vitamin A from vitamin A acetate; 300,000 IU vitamin D from vitamin D3; 8,000 IU vitamin E from dl-α-tocopherol acetate; 600 mg menadione from menadione nicotinamide bisulfite; 6 mg B12 from cyanocobalamin; 9000 mg niacin from niacinamide; 5000 pantothenic acid from d-calcium pantothenate; and 1,500 mg riboflavin from crystalline riboflavin per lb of premix.

[3] Provided 0.035 g chromium, 750,000 IU vitamin A from vitamin A acetate; 4,000 IU vitamin E from dl-α-tocopherol acetate; 40 mg biotin, 400 mg folic acid, 180 mg pyridoxine, 100,000 mg choline, and 9,000 mg carnitine per lb of add pack.

[4] Ronozyme HiPhos (GT) 2700 (DSM Nutritional Products, Parsippany, NJ) provided 1,102,300 phytase units (FTU)/kg of product with a release of 0.10% available P.

Table 2. Efficacy of chemical mitigants used to treat swine feed on PEDV and PRRSV detection using qRT-PCR[1]

Product:	Positive control[2]	Formaldehyde[3]	MCFA[4]	Furst protect[5]			Furst Strike[5]			Prototype A[5]		
Product concentration:	N/A	0.325%	0.50%	0.25%	0.50%	1.00%	0.25%	0.50%	1.00%	0.25%	0.50%	1.00%
PEDV												
qRT-PCR Ct[6]	31.2[b]	34.2[a]	33.5[a]	31.3[b]	30.5[b]	31.4[b]	31.5[b]	30.8[b]	31.7[b]	31.1[b]	30.7[b]	31.0[b]
PRRSV												
qRT-PCR Ct	30.0[c]	42.0[a (2/3)]	34.2[b]	30.2[c]	30.3[c]	30.6[c]	29.6[c]	30.0[c]	30.3[c]	30.5[c]	30.4[c]	31.2[c]

[1] An initial tissue culture (2.5 mL of diluted PEDV or PRRSV, 10^7 TCID$_{50}$/mL) was inoculated into 22.5 g of swine feed. Samples were stored at room temperature for 24 h post-inoculation, then 100 mL of PBS was added, samples were then stored overnight at 39.2°F before PCR analysis.

[2] Positive control: non-chemically treated feed inoculated with virus.

[3] Sal CURB (Kemin Industries, Des Moines, IA) was included in liquid form at the label dosage levels 0.325%.

[4] MCFA treatment was added in liquid form and consisted of a 1:1:1 blend of C6:C8:C10 (hexanoic, octanoic, and decanoic acids, respectively; Sigma Aldrich, St. Louis, MO).

[5] Furst protect, Furst Strike, and Prototype A were added to the diets in dry form.

[6] Cycle threshold (Ct) required to detect viral nucleic acid. A high Ct value indicates less viral nucleic acid present.

[a,b] Means with differing superscripts differ $P < 0.05$ within row.

[(x/x)] Superscripts denote number of samples containing no detectable PEDV genetic material following 45 cycles. A value of 45.0 was assumed for samples with no detectable RNA for analysis.

MCFA = medium chain fatty acid. PEDV = porcine epidemic diarrhea virus. PRRSV = porcine reproductive and respiratory syndrome virus. qRT-PCR = quantitative real time reverse transcription polymerase chain reaction.