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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

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Abstract

Feed and feed ingredients have been shown to be potential vectors of porcine epidemic diarrhea virus (PEDV). Potential strategies to mitigate the risk of disease transmission via feed and feed ingredients would be valuable to the swine and feed milling industries. Therefore, the objective of this experiment was to determine the impact of VevoVitall (5,000 ppm; DSM Nutritional Products Inc., Parsipanny, NJ), CRINA (200 ppm; DSM Nutritional Products Inc., Parsipanny, NJ), and a combination of both products (COMBINATION; 5,000 ppm VevoVitall and 200 ppm CRINA) as feed additives with potential to mitigate the risk of PEDV, in swine gestation diet (FEED) and spraydried porcine plasma (SDPP) as determined by real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analyzed at seven sampling days post laboratory inoculation (d 0, 1, 3, 7, 14, 21, and 42) and bioassay. There was a marginally significant treatment × feed matrix × day interaction ($P = 0.082$), in which the cycle threshold (Ct) value increased over time in the diet when treated with the COMBINATION, whereas, there was no increase over time observed in SDPP. There was a highly significant ($P < 0.001$) feed matrix × day interaction in which the Ct increased over time in FEED, whereas, there was very little increase over time observed in SDPP. Additionally, there was a marginally significant treatment × feed matrix interaction ($P = 0.079$). Overall, the COMBINATION was most effective at reducing the quantity of genetic material as detected by qRT-PCR ($P < 0.001$). Virus shedding was observed in the d 7 post-inoculation SDPP COMBINATION treatment, as well as d 0 FEED COMBINATION treatment. No other treatment bioassay room had detectable RNA shed and detected in fecal swabs or cecal contents (d 1, 3, 7, 14, and 21 post-laboratory inoculation FEED, COMBINATION).

In summary, the combination of CRINA and VevoVitall enhanced degradation of PEDV RNA in swine feed, but had no impact on RNA degradation in SDPP. Furthermore, both untreated feed and feed treated with the combination of CRINA and VevoVitall caused infection at d 0 post-laboratory inoculation; however, neither set of samples was infective at d 1 post-laboratory inoculation.

Keywords

feed additive, feed matrix, PEDV, swine

Cover Page Footnote

Appreciation is expressed to Dr. Charles Farenholtz (Phibro Animal Health, Teaneck, NJ) for technical support and use of facilities and equipment, Dr. Dick Hesse and Joe Anderson for technical support and laboratory use, Elizabeth Poulsen, and Rusty Ransbrough for technical support and laboratory use, Dr. Joe Crenshaw (APC Functional Proteins, Ankeny, IA), as well as Marut Saensukjaroenphon and Mary Muckey for technical support. Appreciation is expressed to DSM Nutritional Products, Inc. (Parsippany, NJ) for project funding.

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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[1,2]

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Summary

Feed and feed ingredients have been shown to be potential vectors of porcine epidemic diarrhea virus (PEDV). Potential strategies to mitigate the risk of disease transmission via feed and feed ingredients would be valuable to the swine and feed milling industries. Therefore, the objective of this experiment was to determine the impact of VevoVitall (5,000 ppm; DSM Nutritional Products Inc., Parsipanny, NJ), CRINA (200 ppm; DSM Nutritional Products Inc., Parsipanny, NJ), and a combination of both products (COMBINATION; 5,000 ppm VevoVitall and 200 ppm CRINA) as feed additives with potential to mitigate the risk of PEDV, in swine gestation diet (FEED) and spray-dried porcine plasma (SDPP) as determined by real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analyzed at seven sampling days post laboratory inoculation (d 0, 1, 3, 7, 14, 21, and 42) and bioassay. There was a marginally significant treatment × feed matrix × day interaction ($P = 0.082$), in which the cycle threshold (Ct) value increased over time in the diet when treated with the COMBINATION, whereas, there was no increase over time observed in SDPP. There was a highly significant ($P < 0.001$) feed matrix × day interaction in which the Ct increased over time in FEED, whereas, there was very little increase over time observed in SDPP.

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[2] Appreciation is expressed to DSM Nutritional Products, Inc. (Parsippany, NJ) for project funding.

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Additionally, there was a marginally significant treatment × feed matrix interaction ($P = 0.079$). Overall, the COMBINATION was most effective at reducing the quantity of genetic material as detected by qRT-PCR ($P < 0.001$). Virus shedding was observed in the d 7 post-inoculation SDPP COMBINATION treatment, as well as d 0 FEED COMBINATION treatment. No other treatment bioassay room had detectable RNA shed and detected in fecal swabs or cecal contents (d 1, 3, 7, 14, and 21 post-laboratory inoculation FEED, COMBINATION).

In summary, the combination of CRINA and VevoVitall enhanced degradation of PEDV RNA in swine feed, but had no impact on RNA degradation in SDPP. Furthermore, both untreated feed and feed treated with the combination of CRINA and VevoVitall caused infection at d 0 post-laboratory inoculation; however, neither set of samples was infective at d 1 post-laboratory inoculation.

Key words: feed additive, feed matrix, PEDV, swine

Introduction

Feed and feed ingredients have been shown to be potential vectors of Porcine Epidemic Diarrhea virus (PEDV).[7,8] Therefore, potential strategies to mitigate the risk of disease transmission via feed and feed ingredients would be valuable to the swine and feed manufacturing industries. Research has been conducted assessing potential mitigation techniques, such as the use of certain feed additives or thermal processing during pelleting of diets. During the pelleting of complete swine diets, previous research has shown that a pelleting conditioner temperature of 130°F was effective at minimizing the risk of PEDV transfer.[9] The application of certain feed additives, including medium chain fatty acids, essential oils, organic acids, and formaldehyde, has been effective at degrading PEDV genetic material in complete feed and feed ingredients as quantified by quantitative real-time polymerase chain reaction (qRT-PCR), but lack of infectivity has not been verified via bioassay.[10]

CRINA and VevoVitall are two commercially available products sold by DSM Nutritional Products (Parsipanny, NJ). CRINA is a combination of essential oils designed to stimulate gut health in swine, and VevoVitall is a 99.9% benzoic acid product designed

[7] Dee, S., T. Clement, A. Schelkopf, J. Nerem, D. Knudsen, J. Christopher-Hennings, and E. Nelson. 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naive pigs following consumption via natural feeding behavior: Proof of concept. BMC Veterinary Research. 10(176).

[8] Pillatzki, A. E., P. C. Gauger, D. M. Madson, E. R. Burrough, JianQiang Zhang, Q. Chen, D. R. Magstadt, P. H. E. Arruda, G. W. Stevenson, and K. J. J. Yoon. 2015. Experimental inoculation of neonatal piglets with feed naturally contaminated with porcine epidemic diarrhea virus. Journal of Swine Health and Production. 23(6): 317-320.

[9] Cochrane, R. A., S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, R. A. Hesse, JianQiang Zhang, M. D. Tokach, J. Bai, and C. K. Jones. 2015. Evaluating chemical mitigation of Porcine Epidemic Diarrhea Virus (PEDV) in swine feed and ingredients. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

[10] Cochrane, R. A., L. L. Schumacher, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. Bai, Q. Chen, JianQiang Zhang, P. C. Gauger, R. G. Main, and C. K. Jones. 2015. Effect of thermal mitigation on Porcine Epidemic Diarrhea Virus (PEDV)-contaminated feed. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

to reduce activity of microorganisms in feed, including fungi, yeasts and certain bacteria, such as *E. coli* and *Salmonella*.[11] However, neither CRINA nor VevoVitall have been tested as potential PEDV mitigants. Therefore, the objective of this experiment was to determine the impact of VevoVitall and CRINA as feed additives with the potential to mitigate PEDV contamination of feed and spray-dried porcine plasma as determined by real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and bioassay.

Procedures

Treatment structure was designed in a 2 × 2 × 2 factorial arrangement with two feed matrices (FEED and SDPP) and feed additive treatment factors including VevoVitall (5,000 ppm; DSM Nutritional Products Inc., Parsippany, NJ) and CRINA (200 ppm; DSM Nutritional Products Inc., Parsippany, NJ), and combination of both products (COMBINATION; 5,000 ppm VevoVitall and 200 ppm CRINA). The swine diet (Table 1) used in this experiment was manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS, and was verified to be devoid of PEDV and porcine delta-coronavirus (PDCoV) ribonucleic acid (RNA) as determined via qRT-PCR prior to initiation of the experiment. Spray-dried porcine plasma (APC Functional Proteins, Ankeny, IA) was also verified to be free of both PEDV and delta-coronavirus RNA prior to use as verified by qRT-PCR.

Feed Additive Treatment

Prior to treatment of feed matrices with feed additive treatments, a 25.0 g sample of each feed matrix was collected and placed in its appropriate bottle. These samples received no virus, and were the positive control samples reserved for the bioassay portion of the experiment. A benchtop paddle mixer was used as previously described[12] for mixing dry products with FEED. Mixing time was 3 min, as was previously verified with a CV of < 10% using a chloride mixer efficiency procedure (Quantab; Hach Co., Loveland, CO). A V-mixer (Cross-Flow Blender; Patterson-Kelley Co., East Stroudsburg, PA) was used to mix feed additive treatments with SDPP. A mixer efficiency test was performed using spray-dried bovine plasma, and resulted in a uniform mix with a mix time of 7.0 min (MicroTracer-F; Microtracers Inc., San Francisco, CA).

Following the mixing of feed matrix and corresponding feed additive treatment, 22.5 g of chemically treated feed matrix was sampled and placed in the appropriate bottle (250 mL Nalgene square wide-mouth HDPE; Thermo Fisher Scientific, Waltham, MA) to be inoculated with PEDV and analyzed on seven sampling days post laboratory inoculation (d 0, 1, 3, 7, 14, 21, and 42), with 3 replications of each sampling day/feed additive treatment combination. This process was repeated for each feed matrix × feed additive treatment combination. Both the paddle mixer and V-blender were cleaned between feed additive treatments initially by high-pressure air, then a flush step was performed

[11] DSM Nutritional Products, Inc., Parsippany, NJ; http://www.dsm.com/markets/anh/en_US/products/products-eubiotics/products-eubiotics-vevovitall.html, Accessed 1/13/2016.

[12] Schumacher, L. L., J. C. Woodworth, C. R. Stark, C. K. Jones, R. A. Hesse, R. G. Main, Jianqiang Zhang, P. C. Gauger, S. S. Dritz, and M. D. Tokach. 2015. Determining the minimum infectious dose of Porcine Epidemic Diarrhea Virus (PEDV) in a feed matrix. Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

with either untreated FEED or SDPP for the paddle mixer and V-blender, respectively, followed by a final cleaning with high-pressure air.

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 8 and had an initial concentration of 10^6 $TCID_{50}$/mL. Fifty mL of concentrated inoculum was mixed with 450 mL of tissue culture media, resulting in a diluted inoculum 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 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 sec to mix the virus evenly within each bottle.

Real-time PCR analysis

Separate bottles were analyzed on d 0, 1, 3, 7, 14, 21, and 42 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 sec, at which point they were allowed to settle overnight at 39.2°F. The following day, supernatant was pulled and aliquoted for further analysis. A total of 4 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 three samples per bottle were stored at -112°F until transported to Iowa State University for the initiation of the bioassay portion of the experiment.

After collection of d 42 post-laboratory inoculation aliquots, qRT-PCR was conducted on designated preserved aliquots at Kansas State University Veterinary Diagnostic Laboratory Molecular Diagnostics Lab as previously described.[12] Fifty 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 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.

Bioassay

A bioassay was performed using selected treatment × time combinations at Iowa State University Veterinary Diagnostic Laboratory to determine the viral infectivity characteristics following protocols previously described by Pillatzki et al., 2015[13] and Thomas et al., 2015.[14]

The experimental protocol for the pig bioassay portion of the experiment was reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee. Forty-eight 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. Upon arrival, piglets were ear tagged, weighed, and administered a dose of cefitiofur (Excede, Zoetis, Florham Park, NJ). Also upon arrival, fecal swabs were obtained and confirmed negative for PEDV, porcine delta coronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV) using a qRT-PCR assay. To further confirm PEDV negative status, serum was collected and confirmed negative for PEDV antibody by an indirect fluorescent antibody (IFA) assay and TGEV antibody by enzyme-linked immunosorbent assay (ELISA) 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.

Briefly, pigs from each experimental treatment were housed in separate rooms with independent ventilation systems. Rooms had solid flooring that was minimally rinsed to reduce risk of PEDV aerosolization. Pigs were fed liquid milk replacer twice daily and offered a commercial pelleted swine diet ad libitum with free access to water. Each pig was administered 10 mL of the PBS supernatant treatment by orogastric gavage using an 8-gauge French catheter 0 d post-bioassay inoculation (dpi).

Rectal swabs were collected on d -2, 0, 2, 4, and 6 dpi from all piglets and tested for PEDV RNA via qRT-PCR. Fresh small intestine, cecum, and colon were collected at necropsy at 7 dpi, along with an aliquot of cecal content. One section of formalin-fixed proximal, middle, and distal jejunum and ileum were collected for histopathology. Cecal content was evaluated for PEDV via qRT-PCR. Tissue was routinely processed and fixed in neutral buffered formalin, embedded, sectioned, and stained with hematoxylin and eosin stain. One section of proximal, middle, and distal jejunum; and three serial sections from the piece of ileum (for a total of six sections of intestine) were evaluated by a veterinary pathologist blind to the treatments. Morphology and IHC data were excluded from the current report.

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[13] Pillatzki, A. E., P. C. Gauger, D. M. Madson, E. R. Burrough, Zhang JianQiang, Q. Chen, D. R. Magstadt, P. H. E. Arruda, G. W. Stevenson, and K. J. J. Yoon. 2015. Experimental inoculation of neonatal piglets with feed naturally contaminated with porcine epidemic diarrhea virus. Journal of Swine Health and Production. 23(6): 317-320.

[14] Thomas, J. T., Qi Chen, P. C. Gauger, L. G. Gimenez-Lirola, Avanti Sinha, K. M. Harmon, D. M. Madson, E. R. Burrough, D. R. Magstadt, H. M. Salzbrenner, M. W. Welch, Yoon Kyoung-Jin, J. J. Zimmerman, and Zhang Jian Qiang. 2015. Effect of porcine epidemic diarrhea virus infectious doses on infection outcomes in naive conventional neonatal and weaned pigs. PLOS ONE. 10(10): e0139266.

Statistical Analysis

Data were analyzed using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) to determine the main effects of feed additive treatment, feed matrix, as well as day post-laboratory inoculation and all associated interactions on PEDV Ct values with individual sample bottle as the experimental unit. Bottle within treatment was included in the model as the subject of the repeated measure of day after laboratory inoculation. Bottle was included in the statistical model as a random effect. Results for the response criteria were considered significant at $P \leq 0.05$ and marginally significant from $P > 0.05$ to $P \leq 0.10$.

Results and Discussion

Quantity of Detectable Viral RNA

There was a marginally significant treatment × feed matrix × day interaction ($P = 0.082$, Table 2) in which the combination of CRINA and VevoVitall resulted in a reduction of quantifiable RNA on d 21 and 42 at a greater rate in feed than in the SDPP matrix. There was a significant ($P < 0.001$, Table 2) feed matrix × day interaction in which the Ct value increased over time in gestation diet, whereas there was very little increase over time observed in SDPP. Additionally, there was a marginally significant treatment × feed matrix interaction ($P = 0.079$, Table 3) in which the combination of CRINA and VevoVitall was more effective at reducing the amount of quantifiable RNA in FEED relative to no feed additive treatment or feed additives included individually, and was no different than untreated or treatment with CRINA or VevoVitall individually in the SDPP matrix. There was no treatment × day interaction ($P = 0.234$). All main effects were highly significant, including treatment, day, and feed matrix ($P \leq 0.003$, Tables 2 and 3). Overall, the combination of CRINA and VevoVitall was most effective at reducing the quantity of genetic material ($P < 0.001$, Ct = 33.0; Table 3), regardless of feed matrix or day post-inoculation. All three feed samples treated with the COMBINATION did not have detectable PEDV RNA at d 42 post-laboratory inoculation, and two samples did not have detectable virus at d 21 post-laboratory inoculation. Cochrane et al. (2015) observed increased efficacy at reducing the amount of quantifiable RNA in complete swine diet and blood meal as the duration of the study progressed using a 2% essential oil blend (garlic oleoresin, turmeric oleoresin, capsicum oleoresin, rosemary extract, and wild oregano essential oils). The maximum efficacy was 14 d post-inoculation and beyond in blood meal and beyond 21 d post-inoculation in the complete swine diet. In the current study, there was no difference in quantification of genetic material among the untreated control, CRINA, and VevoVitall treatments ($P > 0.10$; Ct = 31.8, 31.8, 31.9, respectively). Overall, a greater quantity of PEDV RNA was detected in SDPP relative to feed ($P < 0.001$, Ct = 29.3 ± 0.28 vs. 35.0 ± 0.28, SDPP vs. feed, respectively). The PEDV Ct increased between d 0, 1, 3, 21, and 42 post-laboratory inoculation ($P < 0.001$; 29.3, 30.7, 31.6, 33.9, and 35.2, respectively). There was no difference in Ct between d 3, 7, and 14 post-laboratory inoculation ($P > 0.05$, 31.6, 32.1, and 32.2, respectively).

Infectivity

Upon completion of PCR testing, sixteen samples were strategically selected for assessment of virus infectivity via a bioassay at Iowa State University. The samples selected were d 0 negative control, 7 positive control, and 7 combination of CRINA

and VevoVitall samples. Each sample consisted of 3 supernatant aliquots that each were gavaged into a single pig within bioassay room. Six combinations were selected using swine feed and the combination of CRINA and VevoVitall (d 0, 1, 3, 7, 14, and 21 post-laboratory inoculation) and an additional set of samples was selected using the combination of CRINA and VevoVitall 7 d after inoculation in SDPP. Positive control samples included untreated FEED and SDPP samples at d 0, 3, and 21 post-laboratory inoculation as well as d 1 FEED positive control for a total of 7 total positive control bioassay rooms. The d 0 and d 1 FEED positive control samples were from the current study, however the other 5 positive control samples were in conjunction with additional research from our laboratory using identical procedures in which bioassay controls were shared across projects (Ct = 29.4, 34.1, 31.6, 37.3, 37.8; d 0 SDPP, d 3 FEED, d 3 SDPP, d 21 FEED, d 21 SDPP, respectively).

No PEDV RNA was detected in fecal swabs prior to initiation of the bioassay, and negative control pigs remained negative for PEDV genetic material for the full length of the bioassay as assessed by fecal swabs and cecal content collected at necropsy (Table 4). Genetic material was detected in all positive control FEED pigs beginning at 2 dpi, and viral shedding was observed for the duration of the bioassay. All d 0 post-laboratory inoculation SDPP positive control pigs were shedding PEDV RNA throughout the bioassay, and cecal contents were positive for PEDV RNA at necropsy. No d 1 FEED positive control pigs had detectable RNA in fecal swabs or cecal contents in the second bioassay. All three d 3 post-laboratory inoculation SDPP positive control pigs began shedding virus at 2 dpi, whereas the d 3 post-laboratory inoculation FEED positive controls had no detectable RNA in fecal swabs throughout the bioassay or cecal content at necropsy. No d 21 post-laboratory inoculation positive control pigs had detectable virus in fecal swabs or cecal contents. Thus, pigs became infected with PEDV with both FEED and SDPP at d 0 post-laboratory inoculation, as well as d 3 post-laboratory inoculation in SDPP.

The d 0 FEED combination of CRINA and VevoVitall pigs (3/3) were shedding PEDV RNA as detected by fecal swabs beginning on 2 dpi and remained infected through necropsy at 7 dpi. Virus shedding was observed in fecal swabs in one d 7 post-bioassay inoculation SDPP COMBINATION pig 2 dpi, and all three pigs were shedding virus at 6 dpi and had virus detectable in cecal contents at necropsy. None of the COMBINATION treated FEED had detectable RNA in fecal swabs or cecal contents with the exception of d 0 post-laboratory inoculation samples.

In summary, the combination of CRINA and VevoVitall enhanced degradation of PEDV RNA in swine feed, but had no impact on RNA degradation in SDPP. Furthermore, both untreated feed and feed treated with the combination of CRINA and VevoVitall resulted in PEDV infection at d 0 post-laboratory inoculation; however, neither set of samples were infective at d 1 post-laboratory inoculation.

Table 1. Diet composition (as-fed basis)[1]

Item	Swine gestation diet
Ingredient, %	
Corn	80.40
Soybean meal, 46.5% CP	15.60
Monocalcium phosphate, 21% P	1.40
Calcium carbonate	1.15
Salt	0.50
L-Thr	0.03
Trace mineral premix[1]	0.15
Sow add pack[2]	0.50
Vitamin premix[3]	0.25
Phytase[4]	0.02
Total	100
Calculated analysis, %	
CP	14.1
Crude fiber	2.2
Ether extract	3.0
Ca	0.85
P	0.62
Available P	0.46

[1] Each kilogram contains 26.4 g Mn, 110 g Fe, 110 g Zn, 11 g Cu, 198 mg I, and 198 mg Se.
[2] Each kilogram contains 110,000 mg choline, 44 mg biotin, 330 mg folic acid, 990 mg pyridoxine.
[3] Each kilogram contains 4,400,000 IU vitamin A, 660,000 IU vitamin D3, 17,600 IU vitamin E, 1,760 mg menadione, 3,300 mg riboflavin, 11,000 mg pantothenic acid, 19,800 mg niacin, 15.4 mg vitamin B12.
[4] HiPhos 2700, DSM Nutritional Products, Parsippany, NJ.

Table 2. Interactive means of VevoVitall and/or CRINA, matrix, and day, matrix by day interaction, and main effect of day on PEDV detection as determined by qRT-PCR[1]

	qRT-PCR Ct, Day post-inoculation						
Item	0	1	3	7	14	21	42
Matrix × treatment × day[2]							
FEED							
No treatment	29.4	32.5	31.9	35.2	35.8	37.2	39.3$^{(2/3)}$
CRINA	30.0	32.8	33.3	34.1	35.5	37.7	38.3
VevoVitall	29.8	31.7	33.5	33.4	35.6	38.0	40.4$^{(2/3)}$
CRINA + VevoVitall	30.2	32.4	33.6	36.0	35.5	42.6$^{(1/3)}$	45.0$^{(0/3)}$
SDPP							
No treatment	28.7	29.5	29.7	29.1	28.9	28.3	29.4
CRINA	28.4	29.3	29.3	29.1	28.2	30.3	29.4
VevoVitall	28.8	28.6	30.5	28.8	29.0	28.5	30.2
CRINA + VevoVitall	29.1	29.1	31.1	30.7	29.2	28.3	29.7
Matrix × day[3]							
FEED	29.8^{e,f}	32.3^{d}	33.1^{d}	34.7^{c}	35.6^{c}	38.9^{b}	40.7^{a}
SDPP	28.8^{f}	29.1^{e,f}	30.2^{e}	29.4^{e,f}	28.8^{f}	28.9^{f}	29.7^{e,f}
Day[4]	29.3^{e}	30.7^{d}	31.6^{c}	32.1^{c}	32.2^{c}	33.9^{b}	35.2^{a}

[1] An initial tissue culture (2.5 mL diluted virus inoculum, 10^5 $TCID_{50}$/mL) was inoculated into 22.5 grams of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 200 ppm CRINA, 5,000 ppm VevoVitall, combination of CRINA and VevoVitall (COMBINATION) (DSM Nutritional Products, Parsippany, NJ), or no feed additive treatment. Values are represented by mean quantified PEDV RNA cycle threshold (Ct) value as determined by qRT-PCR.
$^{(X/X)}$ Superscripts denote number of samples with cycle threshold for PEDV RNA below detectable limit of Ct = 45. A value of 45.0 was used for assumed for samples with non-detectable RNA for analysis.
[2] Matrix × treatment × day interaction, $n = 3$ for each value. SEM = 0.90 cycle threshold, $P = 0.082$.
[3] Matrix × day interaction, $n = 12$ for each value. SEM = 0.50 cycle threshold, $P < 0.001$.
[4] Main effect of day, $n = 24$ for each value. SEM = 0.38 cycle threshold, $P < 0.001$.
^{abcdef} Means within interaction or effect lacking a common superscript differ ($P < 0.05$).

Table 3. Interactive means of feed matrix and treatment, and main effect of treatment on PEDV detection using qRT-PCR[1,2]

Item	Control	CRINA	VevoVitall	CRINA + VevoVitall	SEM	*P* =
Matrix × treatment						
FEED[3]	34.5^{b}	34.5^{b}	34.6^{b}	36.5^{a}	0.37	0.079
SDPP[4]	29.1^{c}	29.1^{c}	29.2^{c}	29.6^{c}		
Treatment	31.8^{b}	31.8^{b}	31.9^{b}	33.0^{a}	0.28	0.003

[1] An initial tissue culture (2.5 mL diluted virus inoculum, 10^5 $TCID_{50}$/mL) was inoculated into 22.5 grams of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 200 ppm CRINA, 5,000 ppm VevoVitall, combination of CRINA and VevoVitall (COMBINATION) (DSM Nutritional Products, Parsippany, NJ), or no feed additive treatment. A total of 168 samples were used for the analysis with each treatment represented by a mean of $n = 21$ for the matrix × treatment interaction, and $n = 42$ for the main effect of treatment.
[2] Cycle threshold required to detect genetic material. A higher Ct value is indicative of less genetic material present.
[3] Swine gestation diet.
[4] Spray-dried porcine plasma (APC Functional Proteins, Ankeny, IA).
^{a,b,c} Means within interaction or effect lacking common superscript differ ($P < 0.05$).

Table 4. Effects of VevoVitall and/or CRINA as potential porcine epidemic diarrhea virus (PEDV) mitigation strategies on PEDV detection from feed, pig fecal swabs, and cecum contents[1]

	Fecal swabs[2]				Cecum contents
Item	-2 dpi	2 dpi	4 dpi	6 dpi	7 dpi
FEED					
No treatment					
d 0 no virus	- - -	- - -	- - -	- - -	- - -
d 0	- - -	+ + +	+ + +	+ + +	+ + +
d 1	- - -	- - -	- - -	- - -	- - -
d 3	- - -	- - -	- - -	- - -	- - -
d 21	- - -	- - -	- - -	- - -	- - -
CRINA + VevoVitall					
d 0	- - -	+ + +	+ + +	+ + +	+ + +
d 1	- - -	- - -	- - -	- - -	- - -
d 3	- - -	- - -	- - -	- - -	- - -
d 7	- - -	- - -	- - -	- - -	- - -
d 14	- - -	- - -	- - -	- - -	- - -
d 21	- - -	- - -	- - -	- - -	- - -
SDPP					
No treatment					
d 0 no virus	- - -	- - -	- - -	- - -	- - -
d 0	- - -	+ + +	+ + +	+ + +	+ + +
d 3	- - -	+ + +	+ + +	+ + +	+ + +
d 21	- - -	- - -	- - -	- - -	- - -
CRINA + VevoVitall					
d 7	- - -	+ - -	+ + -	+ + +	+ + +

[1]An initial tissue culture 2.5 mL diluted virus inoculum, 10^5 $TCID_{50}$/mL) was inoculated into 22.5 grams of gestation diet (FEED) or spray-dried porcine plasma (SDPP) treated with 200 ppm CRINA, 5,000 ppm VevoVitall, combination of CRINA and VevoVitall (COMBINATION) (DSM Nutritional Products, Parsippany, NJ), or no feed additive treatment. The supernatant from each sample was then collected for pig bioassay on the appropriate day post-laboratory inoculation and preserved until initiation of the 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 initially 10 d old initial BW = 7.9 lb.

[2]Day post-bioassay inoculation.