Kansas Agricultural Experiment Station Research Reports

Volume 10
Issue 6 *Swine Day*

Article 38

2024

Evaluation of a Blend of Phytochemicals and Carboxylic Acid When Complete Feed and Soybean Meal Were Inoculated with Porcine Epidemic Diarrhea Virus, Porcine Reproductive and Respiratory Syndrome Virus, and Seneca Valley Virus 1

Olivia L. Harrison
Kansas State University

Jianfa Bai
Kansas State University, jbai@vet.k-state.edu

Martee Larson
Kansas State University, mglarson@vet.k-state.edu

Recommended Citation

Harrison, Olivia L.; Bai, Jianfa; Larson, Martee; Pogranichniy, Roman M.; Domingues, Francisco; Holcombe, Nicole; Lopez, Othmar; and Jones, Cassandra K. (2024) "Evaluation of a Blend of Phytochemicals and Carboxylic Acid When Complete Feed and Soybean Meal Were Inoculated with Porcine Epidemic Diarrhea Virus, Porcine Reproductive and Respiratory Syndrome Virus, and Seneca Valley Virus 1," *Kansas Agricultural Experiment Station Research Reports*: Vol. 10: Iss. 6. https://doi.org/10.4148/2378-5977.8653

Evaluation of a Blend of Phytochemicals and Carboxylic Acid When Complete Feed and Soybean Meal Were Inoculated with Porcine Epidemic Diarrhea Virus, Porcine Reproductive and Respiratory Syndrome Virus, and Seneca Valley Virus 1

Funding Source

This work was supported by the Anitox Corporation.

Authors

Olivia L. Harrison, Jianfa Bai, Martee Larson, Roman M. Pogranichniy, Francisco Domingues, Nicole Holcombe, Othmar Lopez, and Cassandra K. Jones

K-STATE Research and Extension

SWINE DAY 2024

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2024

Evaluation of a Blend of Phytochemicals and Carboxylic Acid When Complete Feed and Soybean Meal Were Inoculated with Porcine Epidemic Diarrhea Virus, Porcine Reproductive and Respiratory Syndrome Virus, and Seneca Valley Virus 1

Olivia L. Harrison, Jianfa Bai,[1] Martee Larson,[1] Roman M. Pogranichniy,[1] Francisco Domingues,[2] Nicole Holcombe,[2] Othmar Lopez,[2] and Cassandra K. Jones

Summary

Chemical mitigants have been found to decrease virus concentrations in feed and ingredient matrices. Continued research is needed to identify the appropriate inclusion levels and application time for different viruses in these matrices. Therefore, the objective was to evaluate different inclusion levels of a product utilizing a synergistic blend of phytochemicals and carboxylic acid (PCA) when applied either pre- or post-inoculation of porcine epidemic diarrhea virus (PEDV), porcine reproductive and respiratory syndrome virus (PRRSV) and Seneca Valley virus 1 (SVV1) to complete feed or soybean meal. The experiment was designed in a 2×2 factorial with a PCA-based product, (Finio[3], Anitox Corp. Lawrenceville, GA) applied either before virus inoculation (pre-inoculation) or after inoculation (post-inoculation) at either 3.5 or 5.5 lb/ton. On d 0, samples of the respective matrices were weighed in 50 g aliquots and added to 500 mL bottles. The PCA blend was applied to the pre-inoculation samples at their respective inclusion levels and 50 µL each of 1×10^7 $TCID_{50}$/mL PEDV, 1×10^8 $TCID_{50}$/mL PRRSV, and 1×10^8 $TCID_{50}$/mL SVV1 were added to the post-inoculation samples. All bottles were shaken and allowed to sit at room temperature for 24 hours. On d 1, virus was added to the pre-inoculation samples and chemical mitigants were added to the post-inoculation bottles. Half of the samples were immediately processed (0 hr) and the other half were incubated at room temperature for an additional 24 hours (24 hr). Samples were processed and aliquots were analyzed via a triplex PCR assay at Kansas State University Veterinary Diagnostic Laboratory. Cycle threshold and proportion of PCR positive were analyzed using SAS GLIMMIX v 9.4 (SAS, Inc., Cary, NC), with each virus and matrix combination analyzed individually. In both soybean meal and complete feed an application time × inclusion level inter-

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

[2] Anitox Corporation, Lawrenceville, GA

[3] Finio - A blend of propionic acid, trans-2-hexenal (leaf aldehyde) and nonanoic acid (pelargonic acid)

action was only observed for PRRSV at 0 hr, where less PRRSV RNA was detected ($P < 0.05$) in the post-inoculation samples at either 3.5 or 5.5 lb/ton as compared to the pre-inoculation or control samples. For other viruses at 0 hr in complete feed and soybean meal, the post-inoculation samples had less detectable PEDV or SVV1 RNA ($P < 0.05$) than the pre-inoculation samples. As time continued (24 hr), both pre- and post-inoculation samples had less detectable PEDV RNA ($P < 0.05$) than the controls in complete feed. Interestingly, the positive controls had less detectable viral RNA ($P < 0.05$) at 24 hr in soybean meal compared to either the pre- or post-inoculation samples. This effect is hypothesized to reverse as the mitigated samples have a greater contact time. Overall, the use of a PCA-based product reduced viral concentrations in complete feed and had a variable effect when applied to soybean meal. More research is needed to understand the contact time required for viral reduction and the infectivity of these samples at defined contact times.

Introduction

After the outbreak of porcine epidemic diarrhea virus (PEDV) in 2013, research focused on effective methods to mitigate or reduce the risk of viral transmission through feed and raw ingredients. Formaldehyde is a commonly used mitigant,[4] but due to some environmental concerns, other additives must be considered for use in feed and ingredients. Organic acids, including products utilizing a synergistic blend of phytochemicals and carboxylic acids (PCA) are alternate mitigants which have had variable effects when included in swine diets.[4] The application time of these products may also play a role in viral efficacy, especially when mitigant contact time is minimal.[5] However, the relationship between the application time, inclusion level of PCA, and different viruses in feed or ingredient matrices has not been fully elucidated. Therefore, the objective of this study was to evaluate the effect of PCA inclusion when Seneca Valley virus 1 (SVV1), PEDV, and porcine reproductive and respiratory syndrome virus (PRRSV) were included either prior to or after mitigant application in both complete feed and soybean meal.

Procedures

Inoculum information

An equal volume of SVV1 (GenBank: KX7780101.1), PEDV CO-isolate (GenBank KF272920), and PRRSV 1-7-4 (GenBank: PP239061) were used for feed inoculation. The original stock contained 1×10^8 50% tissue culture infectious dose/mL ($TCID_{50}$/mL) SVV1, 1×10^7 $TCID_{50}$/mL PEDV, and 1×10^8 $TCID_{50}$/mL PRRSV. Viruses were individually packaged into 25 mL aliquots, shipped from South Dakota State University to K-State on dry ice, and stored at -112°F. Prior to the start of the experiment, a 25 mL aliquot of each virus was thawed and divided into four 900 µL aliquots to reduce the freezing and thawing of the stock virus. On the day of inoculation, a

[4] Dee, S. A., M. C. Niederwerder, R. Edler, D. Hanson, A. Singrey, R. Cochrane, G. Spronk, and E. Nelson. 2021. An evaluation of additives for mitigating the risk of virus-contaminated feed using an ice-block challenge model. Transboundary and Emerging Diseases 68::833-845. doi: 10.1111/tbed.13749

[5] 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. Porther, J. Anderson, P.C. Gauger, D.R. Magstadt, J. Zhang, B. Bass, T. Karnezos, B. de Rodas, and 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:skaa159. doi: 10.1093/jas/skaa159

900 µL aliquot of each virus was removed from storage and allowed to thaw at room temperature.

Complete feed and soybean meal information

A corn-soybean meal gestation diet was manufactured in meal form at O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. Soybean was also sourced from the O.H. Kruse Feed Technology Innovation Center from the bulk ingredient storage. Prior to inoculation, 50 g of each matrix was weighed and placed in 500 mL high density polyurethane bottles.

Inoculation and mitigant application

The experiment was designed as a 2 × 2 factorial at two different time points, where mitigant application time (pre- or post-inoculation) by inclusion level (3.5 or 5.5 lb/ton) was evaluated either immediately after both the mitigant and virus were combined (0 h) or after 24 h. There were three replicates for each application time × inclusion level sample at each time point.

At the start of the experiment (d 0), bottles with 50 g of complete feed were separated by the time of inoculation (pre- or post-inoculation). The PCA-based product (Finio, Anitox Corp, Lawrenceville, GA) was applied to the pre-inoculation samples at either 3.5 or 5.5 lb/ton inclusion levels; bottles were shaken for approximately 10 seconds to evenly distribute the mitigant. For the post-inoculation samples, 50 µL of each virus was added to the bottle and shaken for approximately 10 seconds to evenly distribute the virus in the feed. Both pre- and post-inoculation bottles were incubated at room temperature for 24 hours. On d 1, 50 µL of each virus was added to the pre-inoculation samples and either 3.5 or 5.5 lb/ton of the PCA-based product was added to the post-inoculation samples. All bottles were shaken for even product or virus distribution. Following the inclusion of either the virus for the pre-inoculation samples or the mitigant for the post-inoculation samples, hour-0 samples were collected. The remaining bottles were stored for an additional 24 hours at room temperature.

The negative control was not inoculated or mitigated. Two positive controls were included, one inoculated on d 0 as the post-inoculation control and the other inoculated on d 1 as the pre-inoculation control. The sample inoculation and mitigant application process was repeated with soybean meal.

Sample processing

Following the respective incubation time for the 0 or 24 h samples, 200 mL of phosphate buffered solution (PBS) was added to each feed/ingredient sample to create a 20% suspension. Feed/ingredient samples were shaken for approximately 10 sec and stored at 39°F overnight. The next day, 25 mL of the supernatant from the feed samples were transferred to fresh 50 mL conical tubes. The supernatant was centrifuged at 4,000 × g for 10 minutes at 46.5°F. Two 300 µL aliquots were retained for PCR analysis and 20 mL was transferred to a fresh 50 mL conical tube for a bioassay.

Quantitative viral analysis

Samples were analyzed for detection of SVV1, PEDV, and PRRSV using a quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR) assay at the Kansas State University Veterinary Diagnostic Laboratory. First, 50 µL of super-

natant was placed in a deep-well plate and RNA was extracted using a Kingfisher Flex magnetic particle processor (Fisher Scientific, Pittsburgh, PA) and a MagMAX-96 Viral Isolation Kit (Life Technologies, Grand Island, NY). The final elution volume was reduced to 60 µL, and extracted RNA was stored at -112°F until analyzed for SVV1, PEDV, or PRRSV using the qRT-PCR triplex assay with a maximum cycle threshold of 45. Results were reported as the number of samples considered positive and the cycle threshold (Ct) below 45 at which either SVV1, PEDV, or PRRSV RNA was detected.

Statistical analysis

The application time × inclusion level interaction and subsequent main effects were analyzed as a completely randomized design using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute Inc., Cary, NC) with sample bottle as the experimental unit. Fixed effects included the mitigant application time (pre- or post-inoculation), the mitigant application level (3.5 or 5.5 lb/ton), and their associated interactions. Data were separated and individually analyzed based on the matrix (complete feed or soybean meal), time of sampling (0 or 24 hr), and virus (SVV1, PEDV, and PRRSV). To estimate the quantity of detectable viral RNA, the Ct of each sample was used. If no viral RNA was detected, samples were assigned a value of 45. Contrast statements were utilized to compare the 0 h and 24 h samples for each mitigant application time × inclusion level comparison. A Kenward-Roger denominator degree of freedom adjustment was used, as well as a Tukey-Kramer multiple comparison adjustment. Results were considered significant at $P \leq 0.05$.

Results and Discussion

Complete feed

As expected, no viral RNA was detected in the negative control samples. An application time × inclusion level interaction was only observed in PRRSV immediately after the PCA-based product and viruses were combined (0 hr), where less PRRSV RNA was detected ($P < 0.05$) in the post-inoculation samples at 5.5 lb/ton compared to the pre-inoculation samples and the positive controls (Table 1). For SVV1 and PEDV at 0 hr less viral RNA was detected in the post-inoculation samples ($P < 0.05$) as compared to the pre-inoculation samples with the positive control as intermediate (Table 2). Interestingly, this effect lessens at 24 hr, as less PEDV RNA is detected ($P < 0.05$) in both the pre- and post-inoculation samples than the positive control (Table 2). The level of PCA inclusion does not affect the quantity of detectable RNA at 0 hours ($P > 0.05$), but at 24 hr, less PEDV RNA is detected ($P < 0.05$) when PCA are included at either 3.5 or 5.5 lb/ton compared to the positive control (Table 3). When PCA are applied pre-inoculation, the quantity of viral RNA is reduced after 24 hours ($P < 0.05$), but only numerical differences are observed when the PCA-based product is applied post-inoculation ($P > 0.05$; Table 4).

Soybean meal

Viral RNA was not detected in the negative control samples. Similar to complete feed, an application time × inclusion level interaction is only observed in PRRSV at 0 hr, in which post-inoculation samples at either 3.5 or 5.5 lb/ton inclusion have less detectable RNA ($P < 0.05$) than either of the controls or the pre-inoculation samples (Table 5). At 0 hr, the post-inoculation samples have less detectable RNA ($P < 0.05$) than the pre-inoculation samples or the control for all viruses. Interestingly, at 24 hr the positive

control has the least detectable RNA ($P < 0.05$) for all virus compared to the pre- or post-inoculation samples (Table 6). These differences are likely to reverse with longer incubation times as the PCA blends has a longer contact time with the viruses. Less SVV1 RNA was detected ($P < 0.05$) when either 3.5 or 5.5 lb/ton PCA were applied at 0 hr compared to the control (Table 7). At 24 hr, greater concentrations of viral RNA were detected in the mitigated samples ($P < 0.05$) as compared to the control (Table 7), which would most likely be reversed as PCA contact time continues. Overall, viral concentrations decreased as the experiment duration increased ($P < 0.05$) for all viruses (Table 8).

The use of such blend of phytochemicals and carboxylic acid had a variable effect when applied for a short time on either complete feed or soybean meal. A greater PCA contact time is likely needed in order to observe a greater reduction in viral concentrations. Future research should extend the mitigant contact time and should evaluate the infectivity of feed or ingredient matrices following the PCA blend application.

Acknowledgement

This work was supported by the Anitox Corporation.

Brand names appearing in this publication are for product information purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Person using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

Table 1. Effect of application time and phytochemical and carboxylic acids (PCA) inclusion level on the presence and relative quantification of Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) RNA in complete feed.[1,2,3,4]

		Pre-inoculation			Post-inoculation				
	Negative control[2]	Positive control	3.5 lb/ton	5.5 lb/ton	Positive control	3.5 lb/ton	5.5 lb/ton	SEM	*P* =
0 hours									
SVV1	45.0 (0/3)	25.1 (3/3)	25.0 (3/3)	24.0 (3.3)	26.2 (3/3)	26.9 (3/3)	27.0 (3/3)	0.82	0.274
PEDV	45.0 (0/3)	30.1 (3/3)	30.0 (3/3)	29.6 (3/3)	31.1 (3/3)	31.4 (3/3)	31.5 (3/3)	0.58	0.480
PRRSV	45.0 (0/3)	35.5^{ab} (3/3)	34.7^{ab} (3/3)	33.7^{b} (3/3)	35.1^{ab} (3/3)	35.8^{ab} (3/3)	36.6^{a} (3/3)	0.70	0.019
24 hours									
SVV1	45.0 (0/3)	27.8 (3/3)	27.6 (3/3)	27.8 (3/3)	28.3 (3/3)	27.5 (3/3)	27.5 (3/3)	0.43	0.457
PEDV	45.0 (0/3)	30.5 (3/3)	31.8 (3/3)	32.2 (3/3)	30.9 (3/3)	31.8 (3/3)	31.7 (3/3)	0.35	0.196
PRRSV	45.0 (0/3)	34.2 (3/3)	36.0 (3/3)	36.4 (3/3)	34.9 (3/3)	35.6 (3/3)	37.5 (3/3)	0.91	0.465

[1] A blend of phytochemical and carboxylic acids were applied to the complete feed either prior to inoculation (pre-inoculation) or after inoculation (post-inoculation). Phytochemical and carboxylic acids were not applied to the positive controls, but due to the different times of inoculation, two positive controls are included in the analysis. A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.

[2] Negative controls were not included in the statistical analysis.

[3] Presence is determined by the total number of samples that were PCR positive over the total number of replicates (n=3).

[4] Blend of phytochemical and carboxylic acids (Finio, Anitox Corporation, Lawrenceville, GA).

[ab] means with differing superscripts within row differ significantly, $P < 0.05$.

Table 2. Effect of application time of phytochemical and carboxylic acids (PCA) on the presence and relative quantification of Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) RNA in complete feed.[1,2,3,4]

	Negative control	Positive control[5]	Pre-inoculation	Post-inoculation	SEM	*P* =
0 hours						
SVV1	45.0 (0/3)	25.6[ab] (6/6)	24.5[b] (6/6)	26.9[a] (6/6)	0.58	0.003
PEDV	45.0 (0/3)	30.6[ab] (6/6)	29.8[b] (6/6)	31.5[a] (6/6)	0.42	0.003
PRRSV	45.0 (0/3)	35.3[ab] (6/6)	34.2[b] (6/6)	36.2[a] (6/6)	0.51	0.005
24 hours						
SVV1	45.0 (0/3)	28.0 (6/6)	27.7 (6/6)	27.5 (6/6)	0.29	0.213
PEDV	45.0 (0/3)	30.7[b] (6/6)	32.0[a] (6/6)	31.8[a] (6/6)	0.25	0.0003
PRRSV	45.0 (0/3)	34.5[b] (6/6)	36.2[ab] (6/6)	36.6[a] (6/6)	0.69	0.022

[1] A blend of phytochemical and carboxylic acids were applied to the complete feed either prior to inoculation (pre-inoculation) or after inoculation (post-inoculation). The means presented are averaged across the inclusion level (3.5 or 5.5 lb/ton). A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.
[2] Negative controls were not included in the statistical analysis.
[3] Presence is determined by the total number of samples that were PCR positive over the total number of replicates (n=3).
[4] Blend of phytochemical and carboxylic acids (Finio, Anitox Corporation, Lawrenceville, GA).
[5] Positive controls are the average of both the prevention and mitigation positive samples.
[ab] means with differing superscripts within row differ significantly, $P < 0.05$.

Table 3. Effect of phytochemical and carboxylic acids (PCA) inclusion level on the relative quantification of Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) RNA in complete feed.[1,2,3]

	Negative control	Positive control	3.5 lb/ton inclusion	5.5 lb/ton inclusion	SEM	*P* =
0 hours						
SVV1	45.0 (0/3)	25.6 (6/6)	25.9 (6/6)	25.6 (6/6)	0.86	0.858
PEDV	45.0 (0/3)	30.6 (6/6)	30.7 (6/6)	30.6 (6/6)	0.61	0.975
PRRSV	45.0 (0/3)	35.3 (6/6)	35.3 (6/6)	35.1 (6/6)	0.72	0.959
24 hours						
SVV1	45.0 (0/3)	28.0 (6/6)	27.5 (6/6)	27.7 (6/6)	0.29	0.222
PEDV	45.0 (0/3)	30.7[b] (6/6)	31.8[a] (6/6)	32.0[a] (6/6)	0.25	0.0004
PRRSV	45.0 (0/3)	34.5[b] (6/6)	35.8[ab] (6/6)	37.0[a] (6/6)	0.63	0.005

[1] A blend of phytochemical and carboxylic acids were applied at either 3.5 or 5.5 lb/ton to complete feed. The means presented are averaged across the time of application (pre- or post-inoculation). A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.
[2] Negative controls were not included in the statistical analysis.
[3] Blend of phytochemical and carboxylic acids (Finio, Anitox Corporation, Lawrenceville, GA).
[ab] means with differing superscripts within row differ significantly, $P < 0.05$.

Table 4. The effect of time when feed samples were inoculated with Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome virus (PRRSV) for the quantity of detectable RNA (Ct) following application of phytochemical and carboxylic acids (PCA incubated for either 1 hour or 24 hours.[1,2]

	SVV1				PEDV				PRRSV			
	0 hours	24 hours	SEM	*P* =	0 hours	24 hours	SEM	*P* =	0 hours	24 hours	SEM	*P* =
Post-inoculation												
Positive	26.2	28.3	0.97	0.097	31.1	30.9	0.76	0.834	35.1	34.9	0.64	0.682
3.5 lb/ton	26.9	27.5	0.46	0.301	31.4	31.8	0.27	0.227	35.8	35.6	0.69	0.780
5.5 lb/ton	27.0	27.5	0.49	0.310	31.5	31.7	0.48	0.774	36.6	37.5	1.34	0.514
Pre-inoculation												
Positive	25.1[b]	27.8[a]	0.66	0.016	30.1	30.5	0.42	0.391	35.5	34.2	0.77	0.159
3.5 lb/ton	25.0[b]	27.6[a]	0.49	0.006	30.0[b]	31.8[a]	0.44	0.014	34.7	36.0	0.65	0.118
5.5 lb/ton	24.0[b]	27.8[a]	0.71	0.006	29.6[b]	32.2[a]	0.34	0.001	33.7[b]	36.4[a]	0.50	0.006

[1] Complete feed was tri-inoculated with SVV1, PEDV, and PRRSV. A blend of phytochemical and carboxylic acids (PCA) was applied at either 3.5 or 5.5 lb/ton post-inoculation (PCA applied after viral inoculation) or pre-inoculation (PCA applied before viral inoculation)
[2] Blend of phytochemical and carboxylic acids (Finio, Anitox Corporation, Lawrenceville, GA).
[ab] means with differing superscripts within row differ significantly for each individual virus, $P < 0.05$.

Table 5. Effect of application time and phytochemical and carboxylic acids (PCA) inclusion level on the presence and relative quantification of Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) RNA in soybean meal.[1,2,3,4]

		Pre-inoculation			Post-inoculation				
	Negative control[2]	Positive control	3.5 lb/ton	5.5 lb/ton	Positive control	3.5 lb/ton	5.5 lb/ton	SEM	*P* =
0 hours									
SVV1	45.0 (0/3)	23.4 (3/3)	24.5 (3/3)	24.5 (3/3)	23.4 (3/3)	26.8 (3/3)	27.1 (3/3)	0.88	0.124
PEDV	45.0 (0/3)	25.7 (3/3)	25.8 (3/3)	26.2 (3/3)	27.0 (3/3)	28.1 (3/3)	28.9 (3/3)	0.49	0.156
PRRSV	45.0 (0/3)	30.0[b] (3/3)	30.4[b] (3/3)	30.6[b] (3/3)	30.6[b] (3/3)	31.2[a] (3/3)	32.7[a] (3/3)	0.39	0.043
24 hours									
SVV1	45.0 (0/3)	30.9 (3/3)	27.3 (3/3)	27.7 (3/3)	30.3 (3/3)	27.5 (3/3)	27.0 (3/3)	0.59	0.483
PEDV	45.0 (0/3)	29.6 (3/3)	28.5 (3/3)	29.1 (3/3)	29.6 (3/3)	28.9 (3/3)	28.8 (3/3)	0.40	0.518
PRRSV	45.0 (0/3)	33.5 (3/3)	32.0 (3/3)	32.6 (3/3)	33.4 (3/3)	32.8 (3/3)	32.5 (3/3)	0.45	0.330

[1] A blend of phytochemical and carboxylic acids were applied to the soybean either prior to inoculation (pre-inoculation) or after inoculation (post-inoculation). Phytochemical and carboxylic acids were not applied to the positive controls, but due to the different times of inoculation, two positive controls are included in the analysis. A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.
[2] Negative controls were not included in the statistical analysis.
[3] Presence is determined by the total number of samples that were PCR positive over the total number of replicates (n=3).
[4] Blend of phytochemical and carboxylic acids (Finio, Anitox Corporation, Lawrenceville, GA).
[ab] means with differing superscripts within row differ significantly, $P < 0.05$.

Table 6. Effect of application time of phytochemical and carboxylic acids (PCA) on the presence and relative quantification of Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) RNA in soybean meal.[1,2,3,4]

	Negative control	Positive control[5]	Pre-inoculation	Post-inoculation	SEM	*P* =
0 hours						
SVV1	45.0 (0/3)	23.4[b] (6/6)	24.5[b] (6/6)	26.9[a] (6/6)	0.34	< 0.0001
PEDV	45.0 (0/3)	26.3[b] (6/6)	26.0[b] (6/6)	28.5[a] (6/6)	0.42	< 0.0001
PRRSV	45.0 (0/3)	30.3[b] (6/6)	30.5[b] (6/6)	32.5[a] (6/6)	0.29	< 0.0001
24 hours						
SVV1	45.0 (0/3)	30.6[a] (6/6)	27.5[b] (6/6)	27.2[b] (6/6)	0.41	< 0.0001
PEDV	45.0 (0/3)	29.6[a] (6/6)	28.8[b] (6/6)	28.8[b] (6/6)	0.27	0.018
PRRSV	45.0 (0/3)	33.4[a] (6/6)	32.3[b] (6/6)	32.6[ab] (6/6)	0.31	0.006

[1] A blend of phytochemical and carboxylic acids were applied to soybean meal either prior to inoculation (pre-inoculation) or after inoculation (post-inoculation). The means presented are averaged across the inclusion level (3.5 or 5.5 lb/ton). A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.
[2] Negative controls were not included in the statistical analysis.
[3] Presence is determined by the total number of samples that were PCR positive over the total number of replicates (n=3).
[4] Blend of phytochemical and carboxylic acids (Finio, Anitox Corporation, Lawrenceville, GA).
[5] Positive controls are the average of both the prevention and mitigation positive samples.
[ab] means with differing superscripts within row differ significantly, $P < 0.05$.

Table 7. Effect of phytochemical and carboxylic acids (PCA) inclusion level on the relative quantification of Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV), and porcine reproductive and respiratory syndrome virus (PRRSV) RNA in soybean meal.[1,2,3]

	Negative control	Positive control	3.5 lb/ton inclusion	5.5 lb/ton inclusion	SEM	*P* =
0 hours						
SVV1	45.0 (0/3)	23.4[b] (6/6)	25.7[a] (6/6)	25.8[a] (6/6)	0.83	0.017
PEDV	45.0 (0/3)	26.3 (6/6)	27.0 (6/6)	27.5 (6/6)	0.75	0.309
PRRSV	45.0 (0/3)	30.3 (6/6)	31.3 (6/6)	31.6 (6/6)	0.59	0.091
24 hours						
SVV1	45.0 (0/3)	30.6[a] (6/6)	27.4[b] (6/6)	27.3[b] (6/6)	0.42	< 0.0001
PEDV	45.0 (0/3)	29.6[a] (6/6)	28.7[b] (6/6)	28.9[ab] (6/6)	0.27	0.014
PRRSV	45.0 (0/3)	33.4[a] (6/6)	32.4[b] (6/6)	32.6[b] (6/6)	0.32	0.010

[1] A blend of phytochemical and carboxylic acids were applied at either 3.5 or 5.5 lb/ton to soybean meal. The means presented are averaged across the time of application (pre- or post-inoculation). A cycle threshold (Ct) value of 45.0 is considered negative with no detectable viral RNA.
[2] Negative controls were not included in the statistical analysis.
[3] Blend of phytochemical and carboxylic acids (Finio, Anitox Corporation, Lawrenceville, GA).
[ab] means with differing superscripts within row differ significantly, $P < 0.05$.

Table 8. The effect of time when soybean meal samples were inoculated with Seneca Valley virus 1 (SVV1), porcine epidemic diarrhea virus (PEDV) and porcine reproductive and respiratory syndrome virus (PRRSV) for the quantity of detectable RNA (Ct) following application of phytochemical and carboxylic acids (PCA) for either 1 hour or 24 hours.[1,2]

	SVV1				PEDV				PRRSV			
	0 hours	24 hours	SEM	*P* =	0 hours	24 hours	SEM	*P* =	0 hours	24 hours	SEM	*P* =
Post-inoculation												
Positive	23.4[b]	30.3[a]	0.77	0.0009	27.0[b]	29.6[a]	0.36	0.002	30.6[b]	33.4[a]	0.64	0.013
3.5 lb/ton	26.8	27.5	0.37	0.135	28.1[b]	28.9[a]	0.26	0.035	32.2[b]	32.8[a]	0.17	0.028
5.5 lb/ton	27.1	27.0	0.73	0.921	28.9	28.8	0.63	0.889	32.7	32.5	0.38	0.633
Pre-inoculation												
Positive	23.4[b]	30.9[a]	1.35	0.005	25.7[b]	29.6[a]	0.55	0.002	30.0[b]	33.5[a]	0.48	0.002
3.5 lb/ton	24.5[b]	27.3[a]	0.38	0.002	25.8[b]	28.5[a]	0.36	0.002	30.4[b]	32.0[a]	0.41	0.015
5.5 lb/ton	24.6[b]	27.7[a]	0.35	0.0009	26.2[b]	29.1[a]	0.42	0.002	30.6[b]	32.6[a]	0.28	0.002

[1] Soybean meal was tri-inoculated with SVV1, PEDV, and PRRSV. A blend of phytochemical and carboxylic acids (PCA) was applied at either 3.5 or 5.5 lb/ton post-inoculation (PCA applied after viral inoculation) or pre-inoculation (PCA applied before viral inoculation).

[2] Blend of phytochemical and carboxylic acids (Finio, Anitox Corporation, Lawrenceville, GA).

[ab] means with differing superscripts within row differ significantly for each individual virus, $P < 0.05$.