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Evaluation of Formaldehyde When Complete Feed and Soybean Meal Were Inoculated with Porcine Epidemic Diarrhea Virus, Porcine Reproductive and Respiratory Syndrome Virus, and Seneca Valley Virus 1

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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 formaldehyde 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 formaldehyde-based product (Termin-8, Anitox Corp. Lawrenceville, GA) applied either before virus inoculation (pre-inoculation) or after inoculation (post-inoculation) at either a 4 or 6 lb/ton. On d 0, samples of the respective matrices were weighed in 50 g aliquots and added to 500 mL bottles. Chemical mitigants were 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 PCR positive were analyzed using SAS GLIMMIX v 9.4 (SAS, Inc., Cary, NC) with each virus and matrix combination analyzed individually. An application time × inclusion level interaction was observed for PEDV at 0 hr and SVV1 and PEDV at 24 hr in complete feed, where less viral RNA ($P < 0.05$) was detected in the post-inoculation samples at either inclusion level as compared to the positive controls. In soybean meal, the same interaction was observed in PEDV

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and PRRSV at 0 hr and SVV1 and PEDV at 24 hr with less detectable RNA observed ($P < 0.05$) in the post-inoculation samples regardless of inclusion level than the pre-inoculation counterparts and the controls. Overall, an application time effect was noticed in each matrix where less RNA was detected in the post-inoculation samples at 0 hr ($P < 0.05$) compared to the pre-inoculation samples and the control, and at 24 hr, both the pre- and post-inoculation samples had less detectable RNA ($P < 0.05$) than the control. Overall, formaldehyde can reduce detectable RNA immediately in both contaminated complete feed and soybean meal, with greater decreases observed as mitigant contact time increases.

Introduction

Since the outbreak of porcine epidemic diarrhea virus (PEDV) in 2013, there has been increased research evaluating the mitigation of feed to reduce viral presence in feed. Formaldehyde is a chemical mitigant that is hypothesized to bind to viral protein and genetic material, which limits the virus' ability to replicate inside the host cell.[3] Inclusion of formaldehyde has decreased PEDV, porcine reproductive and respiratory syndrome virus (PRRSV), and Seneca Valley virus 1 (SVV1) concentrations in complete feed.[4] However, research evaluating the relationship between application time[5] and the inclusion level needed to have the greatest reduction in viral concentrations in different matrices remains unknown. Therefore, the objective of this study was to evaluate the effect of formaldehyde inclusion when SVV1, PEDV, and 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 900 µL aliquot of each virus was removed from storage and allowed to thaw at room temperature.

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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 formaldehyde 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 (4 or 6 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). Formaldehyde (Termin-8, Anitox Corp, Lawrenceville, GA) was applied to the pre-inoculation samples at either 4 or 6 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 4 or 6 lb/ton of formaldehyde 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 hr 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 supernatant 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 a 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 (4 or 6 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. At 0 hours post-application of both the viruses and formaldehyde, there is a formaldehyde application time × inclusion level interaction for PEDV where less detectable RNA ($P < 0.05$) is present when formaldehyde is applied post-inoculation at either 4 or 6 lb/ton (Table 1). An interaction was not observed ($P > 0.05$) for either SVV1 or PRRSV, but for both viruses, less viral RNA was detected ($P < 0.05$) when formaldehyde was applied post-inoculation than when applied prior to viral inoculation (Table 2).

After 24 hours, an application time × inclusion level interaction was observed for both PEDV and SVV1 (Table 1). Similar to 0 h, there was less detectable PEDV RNA ($P < 0.05$) post-inoculation at either inclusion level as compared to formaldehyde application pre-inoculation. For SVV1, applying formaldehyde at 6 lb/ton post-inoculation resulted in less viral RNA ($P < 0.05$) than at 4 lb/ton or at either pre-inoculation inclusion level. While a PRRSV interaction was not observed, the inclusion of formaldehyde, regardless of the time of application (Table 2) or the inclusion level (Table 3), reduced detectable viral RNA ($P < 0.05$) beyond detectable limits compared to the control. Overall, viral RNA decreased between the 0- and 24-hour sampling times ($P < 0.05$; Table 4).

Soybean meal

Viral RNA was not detected in the negative control samples. Immediately after the viruses and the formaldehyde were combined (0 hours), there was less detectable PEDV and PRRSV RNA ($P < 0.05$) when formaldehyde was applied post-inoculation at either inclusion level compared to the positive controls or the pre-inoculation samples (Table 5). An application time × inclusion level was not observed for SVV1 ($P > 0.05$), but less viral RNA was detected ($P < 0.05$) when formaldehyde was applied post-inoc-

ulation as compared to both the positive control and the pre-inoculation application time (Table 6). The inclusion of formaldehyde at either level decreased the quantity of detectable SVV1 RNA ($P < 0.05$) as compared to the positive controls (Table 7).

At 24 hours post-application of both the viruses and formaldehyde, an interaction was observed for both PEDV and SVV1, where the inclusion of 6 lb/ton formaldehyde post-inoculation reduced the quantity of detectable RNA ($P < 0.05$) as compared to the controls, with other application times and inclusion levels being intermediate (Table 5). Less PRRSV RNA was detected when formaldehyde was applied post-inoculation as compared to pre-inoculation ($P < 0.05$; Table 6) and the inclusion of 6 lb/ton resulted in less detectable RNA ($P < 0.05$) as compared to the untreated, positive control (Table 7). Similar to complete feed, less viral RNA was detected at 24 hours as compared to 0 hours (Table 8).

The use of formaldehyde was able to decrease the quantity of viral RNA in both complete feed and soybean meal. Formaldehyde applied after inoculation immediately reduces more RNA than when applied prior to inoculation, but the magnitude of difference lessens after 24 hours. Further research is needed to understand the infectivity of these samples and whether greater differences are noticed between the mitigated and unmitigated samples with longer incubation times.

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Table 1. Effect of application time and formaldehyde 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	4 lb/ton	6 lb/ton	Positive control	4 lb/ton	6 lb/ton	SEM	*P* =
0 hours									
SVV1	45.0 (0/3)	25.1 (3/3)	25.6 (3/3)	25.9 (3/3)	26.2 (3/3)	28.5 (3/3)	29.3 (3/3)	0.74	0.098
PEDV	45.0 (0/3)	30.1^{b} (3/3)	30.4^{b} (3/3)	30.6^{b} (3/3)	31.1^{b} (3/3)	34.4^{a} (3/3)	36.1^{a} (3/3)	0.83	0.007
PRRSV	45.0 (0/3)	35.5 (3/3)	36.6 (3/3)	38.9 (2/3)	35.1 (3/3)	42.7 (1/3)	42.4 (1/3)	2.70	0.265
24 hours									
SVV1	45.0 (0/3)	27.8^{d} (3/3)	30.0^{c} (3/3)	31.4^{bc} (3/3)	28.3^{d} (3/3)	32.6^{b} (3/3)	34.5^{a} (3/3)	0.50	0.007
PEDV	45.0 (0/3)	30.5^{c} (3/3)	34.4^{bc} (3/3)	39.6^{ab} (2/3)	30.9^{c} (3/3)	45.0^{a} (0/3)	45.0^{a} (0/3)	1.64	0.003
PRRSV	45.0 (0/3)	34.2 (3/3)	45.0 (0/3)	45.0 (0/3)	34.9 (3/3)	45.0 (0/3)	45.0 (0/3)	0.35	0.366

[1] Formaldehyde was applied to the complete feed either prior to inoculation (pre-inoculation) or after inoculation (post-inoculation). Formaldehyde was 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] Formaldehyde (Termin-8, Anitox Corporation, Lawrenceville, GA)
[abcd] means with differing superscripts within row differ significantly, $P < 0.05$

Table 2. Effect of application time of formaldehyde 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^{b} (6/6)	25.8^{b} (6/6)	28.9^{a} (6/6)	0.53	< 0.0001
PEDV	45.0 (0/3)	30.6^{b} (6/6)	30.5^{b} (6/6)	35.3^{a} (6/6)	0.64	< 0.0001
PRRSV	45.0 (0/3)	35.3^{b} (6/6)	37.8^{b} (5/6)	42.8^{a} (2/6)	1.76	0.003
24 hours						
SVV1	45.0 (0/3)	28.0^{c} (6/6)	30.7^{b} (6/6)	33.6^{a} (6/6)	0.53	< 0.0001
PEDV	45.0 (0/3)	30.7^{c} (6/6)	37.0^{b} (5/6)	45.0^{a} (0/6)	1.41	< 0.0001
PRRSV	45.0 (0/3)	34.5^{b} (6/6)	45.0^{a} (0/6)	45.0^{a} (0/6)	0.25	< 0.0001

[1] Formaldehyde was 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 (4 or 6 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] Formaldehyde (Termin-8, Anitox Corporation, Lawrenceville, GA)
[5] Positive controls are the average of both the prevention and mitigation positive samples.
[abc] means with differing superscripts within row differ significantly, $P < 0.05$

Table 3. Effect of formaldehyde 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	4 lb/ton inclusion	6 lb/ton inclusion	SEM	*P* =
0 hours						
SVV1	45.0 (0/3)	25.6 (6/6)	27.0 (6/6)	27.6 (6/6)	0.96	0.135
PEDV	45.0 (0/3)	30.6 (6/6)	32.4 (6/6)	33.4 (6/6)	1.36	0.161
PRRSV	45.0 (0/3)	35.4 (6/6)	39.7 (4/6)	40.6 (3/6)	2.14	0.057
24 hours						
SVV1	45.0 (0/3)	28.0[b] (6/6)	31.3[a] (6/6)	32.9[a] (6/6)	0.81	< 0.0001
PEDV	45.0 (0/3)	30.7[b] (6/6)	39.7[a] (3/6)	42.3[a] (2/6)	2.41	0.0006
PRRSV	45.0 (0/3)	34.5[b] (6/6)	45.0[a] (0/6)	45.0[a] (0/6)	0.25	< 0.0001

[1] Formaldehyde was applied at either 4 or 6 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] Formaldehyde (Termin-8, 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 formaldehyde 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
4 lb/ton	28.5[b]	32.6[a]	0.61	<0.0001	34.4[b]	45.0[a]	0.61	<0.0001	42.7	45.0	2.25	0.374
6 lb/ton	29.3[b]	34.5[a]	0.59	<0.0001	36.1[b]	45.0[a]	0.81	0.0004	42.4	45.0	2.60	0.374
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
4 lb/ton	25.6[b]	30.0[a]	0.40	0.0004	30.4[b]	34.4[a]	0.94	0.013	36.6[b]	45.0[a]	0.14	<0.0001
6 lb/ton	25.9[b]	31.4[a]	0.36	0.0001	30.6[b]	39.6[a]	2.73	0.030	38.9	45.0	3.06	0.116

[1] Complete feed was tri-inoculated with SVV1, PEDV, and PRRSV. Formaldehyde was applied at either 4 or 6 lb/ton post-inoculation (formaldehyde applied after viral inoculation) or pre-inoculation (formaldehyde applied before viral inoculation)
[2] Termin-8, 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 formaldehyde 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	4 lb/ton	6 lb/ton	Positive control	4 lb/ton	6 lb/ton	SEM	*P* =
0 hours									
SVV1	45.0 (0/3)	23.4 (3/3)	24.7 (3/3)	24.6 (3/3)	23.4 (3/3)	27.3 (3/3)	27.4 (3/3)	0.89	0.086
PEDV	45.0 (0/3)	25.7^{b} (3/3)	26.3^{b} (3/3)	26.2^{b} (3/3)	27.0^{b} (3/3)	29.9^{a} (3/3)	31.1^{a} (3/3)	0.51	0.001
PRRSV	45.0 (0/3)	30.0^{b} (3/3)	31.2^{b} (3/3)	31.2^{b} (3/3)	30.6^{b} (3/3)	34.4^{a} (3/3)	36.3^{a} (3/3)	0.58	0.0006
24 hours									
SVV1	45.0 (0/3)	30.9^{ab} (3/3)	28.3^{b} (3/3)	28.9^{b} (3/3)	30.3^{ab} (3/3)	30.5^{ab} (3/3)	32.4^{a} (3/3)	0.90	0.020
PEDV	45.0 (0/3)	29.6^{c} (3/3)	30.2^{bc} (3/3)	31.3^{bc} (3/3)	29.6^{c} (3/3)	33.1^{ab} (3/3)	35.5^{a} (3/3)	0.87	0.016
PRRSV	45.0 (0/3)	33.5 (3/3)	34.8 (3/3)	39.1 (2/3)	33.4 (3/3)	42.3 (1/3)	42.5 (1/3)	2.76	0.188

[1] Formaldehyde was applied to soybean meal either prior to inoculation (pre-inoculation) or after inoculation (post-inoculation). Formaldehyde was 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] Formaldehyde (Termin-8, Anitox Corporation, Lawrenceville, GA)

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

Table 6. Effect of application time of formaldehyde 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)	24.6^{b} (6/6)	27.3^{a} (6/6)	0.57	< 0.0001
PEDV	45.0 (0/3)	26.3^{b} (6/6)	26.3^{b} (6/6)	30.5^{a} (6/6)	0.45	< 0.0001
PRRSV	45.0 (0/3)	30.3^{b} (6/6)	31.2^{b} (6/6)	35.4^{a} (6/6)	0.52	< 0.0001
24 hours						
SVV1	45.0 (0/3)	30.6^{a} (6/6)	28.6^{b} (6/6)	31.5^{a} (6/6)	0.68	0.003
PEDV	45.0 (0/3)	29.6^{b} (6/6)	30.7^{b} (6/6)	34.3^{a} (6/6)	0.52	< 0.0001
PRRSV	45.0 (0/3)	33.4^{b} (6/6)	37.0^{b} (5/6)	42.4^{a} (2/6)	1.92	0.001

[1] Formaldehyde was applied to soybean meal either prior to inoculation (pre-inoculation) or after inoculation (post-inoculation). The means presented are averaged across the inclusion level (4 or 6 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] Formaldehyde (Termin-8, 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 formaldehyde 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	4 lb/ton inclusion	6 lb/ton inclusion	SEM	*P* =
0 hours						
SVV1	45.0 (0/3)	23.4[b] (6/6)	26.0[a] (6/6)	26.0[a] (6/6)	0.90	0.014
PEDV	45.0 (0/3)	26.3 (6/6)	28.1 (6/6)	28.7 (6/6)	1.18	0.152
PRRSV	45.0 (0/3)	30.3[b] (6/6)	32.8[ab] (6/6)	33.7[a] (6/6)	1.17	0.026
24 hours						
SVV1	45.0 (0/3)	30.6 (6/6)	29.4 (6/6)	30.6 (6/6)	0.95	0.384
PEDV	45.0 (0/3)	29.6[b] (6/6)	31.6[ab] (6/6)	33.4[a] (6/6)	1.08	0.011
PRRSV	45.0 (0/3)	33.4[b] (6/6)	38.5[ab] (4/6)	40.8[a] (3/6)	2.30	0.017

[1] Formaldehyde was applied at either 4 or 6 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] Formaldehyde (Termin-8, 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 formaldehyde 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	0.001	27.0[b]	29.6[a]	0.36	0.002	30.6[b]	33.4[a]	0.64	0.013
4 lb/ton	27.3[b]	30.5[a]	1.11	0.045	29.9	33.1	1.14	0.052	34.4[b]	42.3[a]	2.82	0.050
6 lb/ton	27.4[b]	32.4[a]	0.91	0.005	31.1[b]	35.5[a]	1.06	0.015	36.3	42.5	2.52	0.069
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
4 lb/ton	24.7[b]	28.3[a]	0.44	0.001	26.3[b]	30.2[a]	0.32	0.0003	31.2[b]	34.8[a]	0.50	0.002
6 lb/ton	24.6[b]	28.9[a]	0.39	0.0004	26.3[b]	31.3[a]	0.20	<0.0001	31.2	39.1	2.93	0.054

[1] Soybean meal was tri-inoculated with SVV1, PEDV, and PRRSV. Formaldehyde was applied at either 4 or 6 lb/ton post-inoculation (formaldehyde applied after viral inoculation) or pre-inoculation (formaldehyde applied before viral inoculation)
[2] Termin-8, Anitox Corporation, Lawrenceville, GA
[ab] means with differing superscripts within row differ significantly for each individual virus, $P < 0.05$