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Evaluating Chemical Mitigation of Porcine Epidemic Diarrhea Virus (PEDV) in Swine Feed and Ingredients¹

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Summary

Porcine Epidemic Diarrhea Virus (PEDV) is primarily transmitted by fecal-oral contamination. Research has confirmed swine feed or ingredients as potential vectors of transmission, so strategies are needed to mitigate PEDV in feed. The objective of this experiment was to evaluate the effectiveness of various chemical additives to prevent or mitigate post-processing PEDV contamination in swine feed and ingredients. Treatments were arranged in a 7 × 4 factorial with seven chemical treatments and four feed matrices. The chemical treatments included: negative control with no chemical addition, 0.3% commercial formaldehyde product, 1% sodium bisulfate, 1% sodium chlorate, 3% custom organic acid blend (OA), 2% custom essential oil blend (EO), and 2% custom medium chain fatty acid blend (MCFA). The four matrices included a complete swine diet, blood meal, meat and bone meal, and spray-dried animal plasma. Matrices were first chemically treated, then inoculated with PEDV, stored at room temperature, and analyzed by RT-PCR on d 0, 1, 3, 7, 14, 21, and 42 post inoculation.

Formaldehyde, MCFA, EO, and OA addition each decreased RNA concentration of PEDV compared to the control ($P < 0.05$), with formaldehyde being the most effective on d 0. Feed matrix appears important in PEDV retention, as RNA concentrations were lower in the swine diet and blood meal than meat and bone meal or spray-dried animal plasma on d 0 ($P < 0.05$). Additionally, PEDV stability over time was influenced by matrix as RNA concentrations were greater by d 42 for spray-dried animal plasma and meat and bone meal than the complete swine diet and blood meal.

In summary, time, formaldehyde, MCFA, EO, and OA all enhance the RNA degradation of PEDV in swine feed and ingredients as measured by RT-PCR, but their effec-

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tiveness varies within matrix. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially-available formaldehyde product.

Key words: PEDV, chemical treatment, feed matrix, swine

Introduction

Porcine Epidemic Diarrhea Virus (PEDV) is an enveloped single-stranded positive-sense RNA virus that was first identified in the United States in May 2013. The coronavirus affects pigs of all life stages, but the highest mortality rates are seen within suckling pigs because of their immature digestive tracts.

The virus is known to be spread by the fecal-oral route, but epidemiological and controlled experiments confirm that complete feed or feed components can be one of the many possible vectors of transmission of PEDV. Viral transmission may occur by direct contamination, but is more likely from cross contamination during the manufacturing, transportation, and storage of feed and ingredients. Viral destruction by thermal processing or irradiation are important to evaluate, but both are point-in-time mitigants that do not offer residual protection from contamination post-processing, which is a solution offered by chemical treatment. Therefore, the objective of this experiment was to evaluate the effectiveness of various chemical additives to prevent or reduce the amount of viral particles of PEDV in swine feed and ingredients that had been contaminated post-processing.

Materials and Methods

Seven chemical treatments were applied to four different feed matrices. The chemical treatments included: 1) negative control with no chemical addition; 2) 0.3% commercial formaldehyde product (Termin-8, Anitox Corp, Lawrenceville, GA); 3) 1% sodium bisulfate (Jones-Hamilton Co, Walbridge, OH); 4) 1% sodium chlorate; 5) 3% OA blend (lactic, propionic, formic, and benzoic); 6) 2% essential oil blend (garlic oleoresin, turmeric oleoresin, capsicum oleoresin, rosemary extract, and wild oregano essential oils); and 7) 2% medium chain fatty acid blend (caproic, caprylic, and capric acids). The four matrices included: 1) complete swine diet; 2) blood meal; 3) porcine meat and bone meal; and 4) spray-dried porcine plasma. The complete swine diet was a grain-based Phase 3 swine nursery diet manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, Kansas. All protein meals were obtained in dried form and untreated with preservatives, antimicrobials, or other chemicals. All feed matrices tested negative for PEDV by RT-PCR prior to chemical treatment. Each feed matrix was placed in a lab scale ribbon mixer. At this stage the liquid chemicals were fogged onto the feed and the powdered treatments were mixed directly into the mixer. All chemical treatments were applied on a wt/wt basis. The dry powder treatments were mixed for three minutes, the EO treatment mixed for 15 minutes because of the known viscosity of the product, and all other liquid treatments were mixed for five minutes.

Once the treatments were mixed, a total of 0.20 lb. of product was collected from 10 different locations and placed into a polyethylene container for inoculation. Between protein meals of the same chemical treatment, the mixer was physically cleaned to re-

move all organic residues. Between different chemical treatments, the mixer was physically and wet cleaned and dried to remove all organic and chemical residues. A ground corn flush between treatments also prevented treatment-to-treatment cross-contamination.

Inoculation

The 28 samples were inoculated in polyethylene containers at the Kansas State University Veterinary Diagnostic Laboratory with USA/IN/2013/19338 Passage 7 grown in Vero cells with an infectious titer of 5.6×10^6 TCID₅₀/ml. A total of 0.33 oz. (0.03 oz. cell fluid + 0.30 oz. cell culture fluid) was added to each 0.20 lb. sample to result in 0.22 lb. of inoculated feed matrix. The 10 mL inoculum was added by two 0.17 oz. additions, and the container was sealed and shaken to distribute virus after each addition. Each of the 28 inoculated matrices were divided into twenty-one 0.006 lb. sub-samples and placed into 0.5 oz. conical tubes (7 days \times 3 reps). Tubes were stored at room temperature until analyzed by Real-Time PCR. There were three replicates per sub-sample. Untreated control supernatant from the untreated controls for each of the four matrices on d 0 was harvested and aliquots frozen to use as controls on each subsequent day's analysis to determine intra- and inter-assay variation. There was very little variation among sampling days or within duplicate, suggesting that the RT-PCR assay was highly sensitive, accurate, and precise (Table 1).

Real-time PCR analysis

Four tenths of an ounce (oz.) of 1 \times PBS (Life Technologies, Grand Island, NY) was added to each three gram sample, vortexed and placed in a 39.2°F refrigerator overnight. The following day 0.03 oz. of supernatant was removed for archiving. Fifty microliters (μ L) of supernatant from each sample was loaded into a deep well plate and extracted using a Kingfisher 96 magnetic particle processor (Fisher Scientific, Pittsburgh, PA) and the MagMAX-96 Viral RNA Isolation kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions with one modification, reducing the final elution volume to 60 μ L. One negative extraction control consisting of all reagents except the sample was included in each extraction, as well as two replicates of an aliquot of the day 0 untreated controls for all sample types. The extracted RNA was frozen at -4°F until assayed by quantitative reverse-transcription PCR (qRT-PCR). Analyzed values represent threshold cycle time (Ct) at which the virus was detected, and thus lower values indicate greater nucleic acid presence, not infectivity.

Results and Discussion

All main effects and interactions were highly significant ($P < 0.001$; Table 2). Overall, the commercial formaldehyde product, MCFA, EO, OA, and sodium chlorate all differed from the control ($P < 0.05$). The commercial formaldehyde was the most effective chemical treatment (32.5 Ct), followed by the MCFA (31.4 Ct) EO (30.5 Ct), and OA treatments (30.4 Ct); all of which improved ($P < 0.05$) the quantity of detectable PEDV nucleic acid compared to the untreated control (29.9 Ct) as detected by RT-PCR (Table 3). Significant differences were also observed between each of the feed ma-

⁶ Dee et al., 2014. An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naïve pigs following consumption via natural feeding behavior: proof of concept. BMC Veterinary Research 2014, 10:176.

trices ($P < 0.05$). Overall, blood meal had the highest PEDV Ct (32.9 Ct), followed by the complete swine diet, spray-dried porcine plasma, and porcine meat and bone meal ($P < 0.05$; 32.0, 29.2, and 28.1 CT, respectively; Table 4). Time also affected PEDV concentration detected by RT-PCR, with d 0 and 1 being statistically similar (29.0 vs. 28.8 CT, respectively; $P > 0.05$), but lower ($P < 0.05$) than d 3 (29.8 CT; Table 5). The Ct increased over time during d 3, 7, 14, and 21 ($P < 0.05$; 29.8, 30.6, 31.1, and 32.1, respectively). However, d 21 and 42 were similar ($P > 0.05$) overall (32.1 vs. 32.3 CT, respectively).

Interactions are presented graphically and provide more relevant results regarding the effects of specific chemical mitigants in various matrices over time. The PEDV Ct in the untreated control of the complete swine diet increased until d 21, after which it remained relatively constant (Fig. 1). Of the tested chemical mitigants in the complete swine diet, the MCFA treatment was the most effective overall, with the EO treatment reaching similar efficacy by d 42. The PEDV Ct in the untreated control of the blood meal was similar to that of the complete swine diet, in that it increased until d 21, but was relatively similar between d 21 and d 42 (Fig. 2). Although the EO treatment was not effective at mitigating PEDV according to RT-PCR through d 7, it was the most effective on d 14, 21, and 42. Interestingly, the PEDV Ct in the untreated control of the porcine meat and bone meal was highly stable throughout the experimental period, with no chemical showing substantial viral detection effects, even though differences were statistically significant (Fig. 3). The PEDV Ct in the untreated control of the spray-dried porcine plasma was also relatively stable over time (Fig. 4). However, the commercial formaldehyde product was highly successful at mitigating PEDV according to RT-PCR in spray-dried porcine plasma compared to other tested chemical additives.

It is interesting to evaluate the untreated controls in each matrix over time to further emphasize that matrix is a factor affecting PEDV Ct according to RT-PCR (Fig. 5). Again, the PEDV Ct in blood meal and complete swine diet increase over time consistently until d 21, but are relatively stable from d 21 to 42. Meanwhile, the porcine meat and bone meal and spray-dried porcine plasma maintain the PEDV Ct more consistently over time.

In summary, time, formaldehyde, MCFA, EO, and OA all enhance the RNA degradation of PEDV in swine feed and ingredients, but their effectiveness varies within matrix. Notably, the MCFA was equally as successful at mitigating PEDV as a commercially-available formaldehyde product.

Table 1. Within-day laboratory controls of PEDV-contaminated samples used to evaluate the interassay variation¹

Item	Day												
	0	1		3		7		14		21		42	
Swine diet	28.2	29.3	28.8	29.1	28.8	29.2	28.6	28.3	28.2	28.8	28.6	28.8	28.6
Blood meal	30.6	31.5	31.3	31.4	31.3	31.5	31.3	31.0	31.0	31.3	31.0	31.1	31.2
Meat and bone meal	26.4	26.2	25.9	26.2	26.2	26.0	26.1	26.0	26.0	26.3	26.2	26.3	26.2
Spray-dried animal plasma	28.2	27.0	26.6	27.3	26.6	27.7	28.1	27.4	27.2	27.3	26.5	26.8	26.7

¹ Values are represented by quantified Ct value. In each case the d 0 samples for the analysis were used for both the interassay variation and results. The d 0 samples also have a mean of N=3. For each of the other analysis days, 2 aliquots for each day were drawn from the d 0 analysis day and frozen until they were used for the appropriate day. In each case the d 1 to 42 interassay variations are represented by a mean of N=2 for each day with each mean shown.

Table 2. Main effects and interactions of treatment, feed matrix, and day on PEDV quantity as detected by RT-PCR.

Effect	P =
Treatment	< 0.001
Feed matrix	< 0.001
Day	< 0.001
Treatment × Feed matrix	< 0.001
Treatment × Day	< 0.001
Feed matrix × Day	< 0.001
Treatment × Feed matrix × Day	< 0.001

Table 3. Main effect of chemical treatment on PEDV detection using RT-PCR¹

Item	Control	Essential oil	Medium chain fatty acids	Organic acids	Sodium bisulfate	Sodium chlorate	Termin-8	SEM	P =
Ct value ²	29.9 ^d	30.5 ^c	31.4 ^b	30.4 ^c	29.7 ^d	29.3 ^c	32.5 ^a	0.08	< 0.0001

¹ A total of 588 samples were used for the analysis with each treatment represented by a mean of N=84.

² Cycle time required to detect the genetic material. A higher Ct value means less genetic material present.

^{abcdc} Means within a row lacking a common superscript differ P < 0.05.

Table 4. Main effects of feed matrix on detection of PEDV using RT-PCR¹

Item	Swine diet	Blood meal	Porcine meat/ bone meal	Spray dried animal plasma	SEM	P =
Ct value ²	32.0 ^b	32.9 ^a	28.1 ^d	29.2 ^c	0.06	< 0.0001

¹ A total of 588 samples were used for the analysis with each treatment represented by a mean of N=147.

² Cycle time required to detect the genetic material. A higher Ct value means less genetic material present.

^{abcd} Means within a row lacking a common superscript differ.

Table 5. Main effect of day post inoculation on detection of PEDV using RT-PCR¹

Item	Day							SEM	P =
	0	1	3	7	14	21	42		
Ct value ²	29.0 ^c	28.8 ^c	29.8 ^d	30.6 ^c	31.1 ^b	32.1 ^a	32.3 ^a	0.08	< 0.0001

¹A total of 588 samples were used for the analysis with each treatment represented by a mean of N=84.

²Cycle time required to detect the genetic material. A higher Ct value means less genetic material present.

^{abcde} Means within a row lacking a common superscript differ.

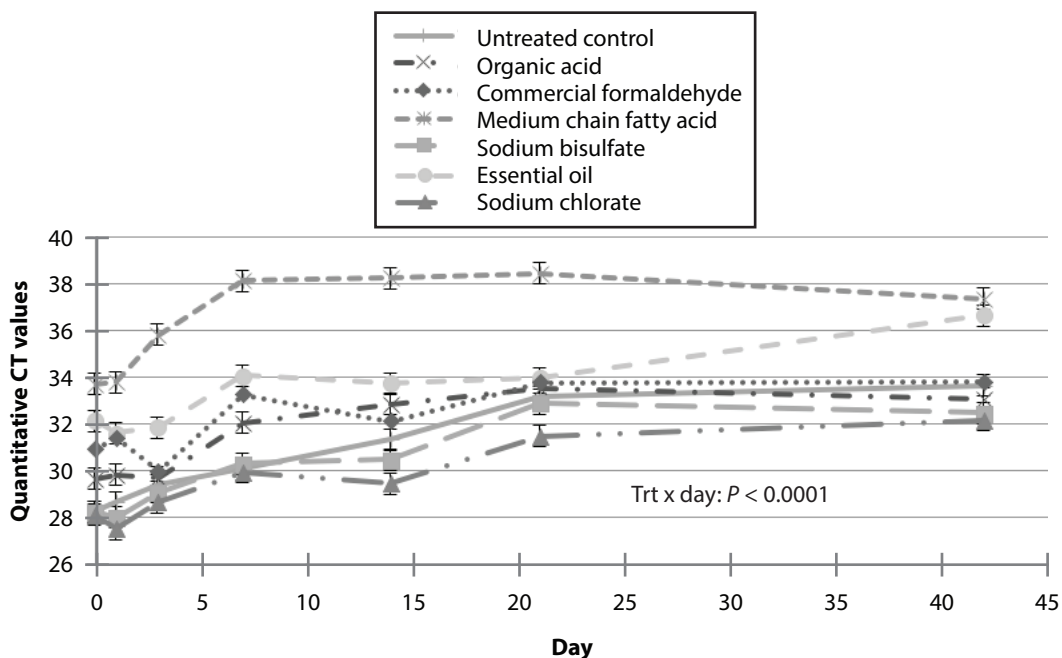


Figure 1. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated complete swine diet stored at room temperature

Data were analyzed by PCR with each data point represented by N=3. The higher the Ct value, the less quantity of PEDV RNA genetic material is detected.

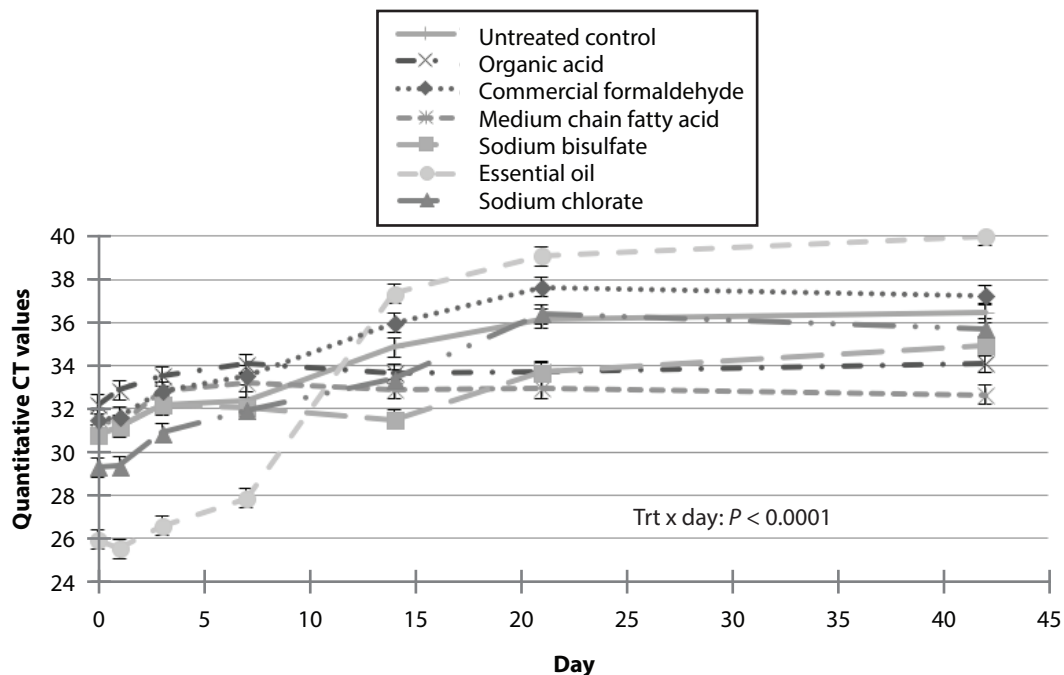


Figure 2. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated blood meal stored at room temperature

Data were analyzed by PCR with each data point represented by N=3. The higher the Ct value, the less quantity of PEDV RNA genetic material is detected.

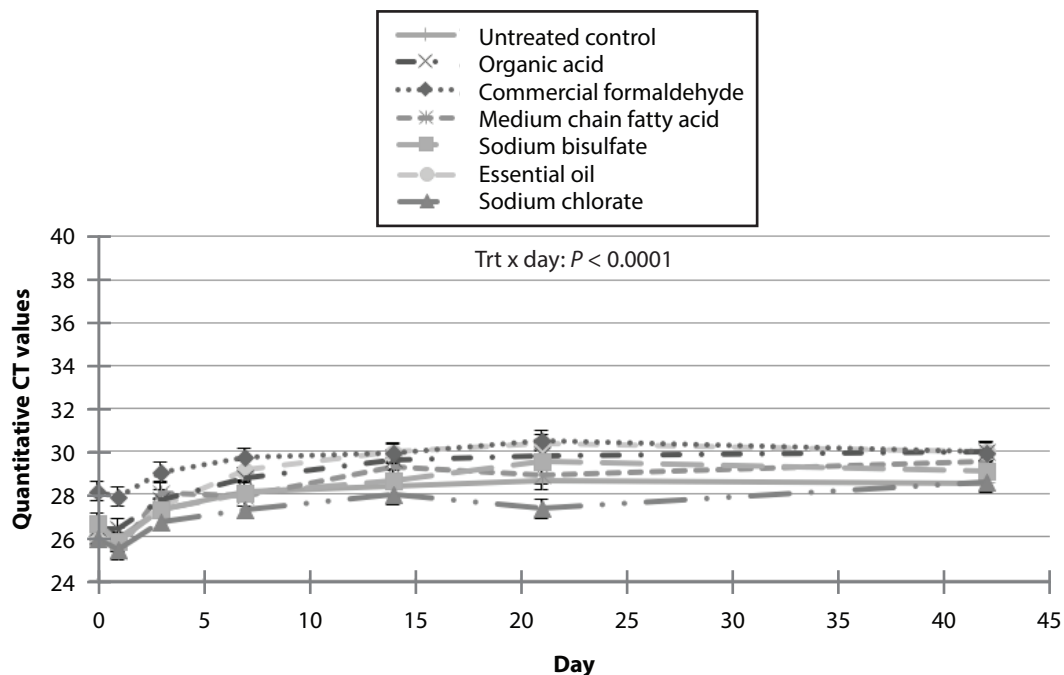


Figure 3. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated meat and bone meal stored at room temperature

Data were analyzed by PCR with each data point represented by N=3. The higher the Ct value, the less quantity of PEDV RNA genetic material is detected.

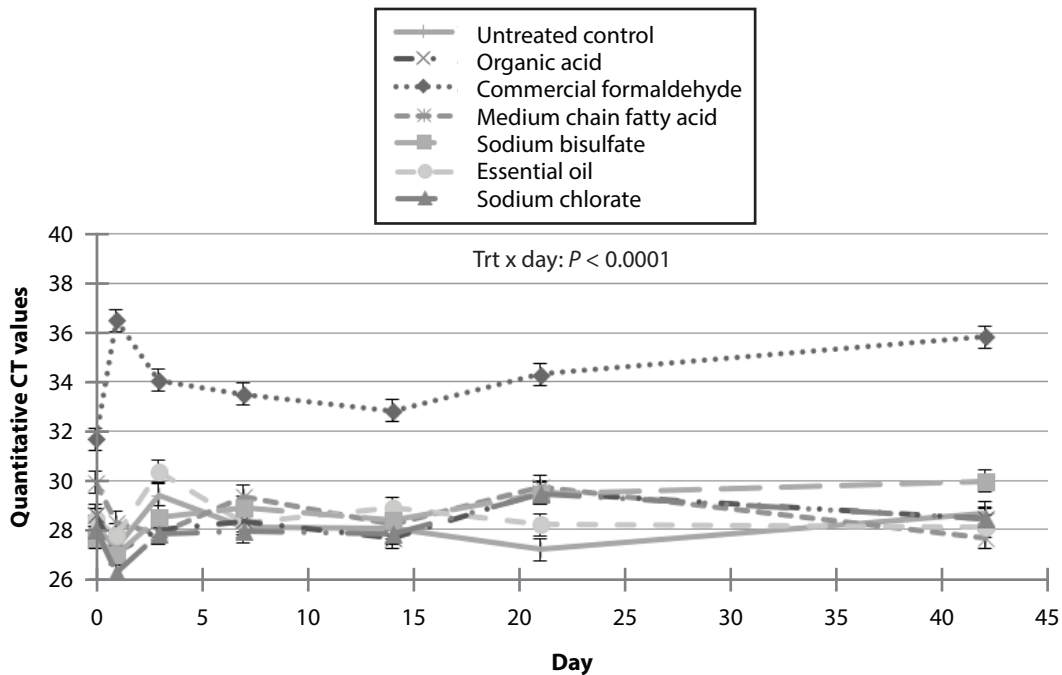


Figure 4. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated spray dried animal plasma stored at room temperature
 Data were analyzed by PCR with each data point represented by N=3. The higher the Ct value, the less quantity of PEDV RNA genetic material is detected.

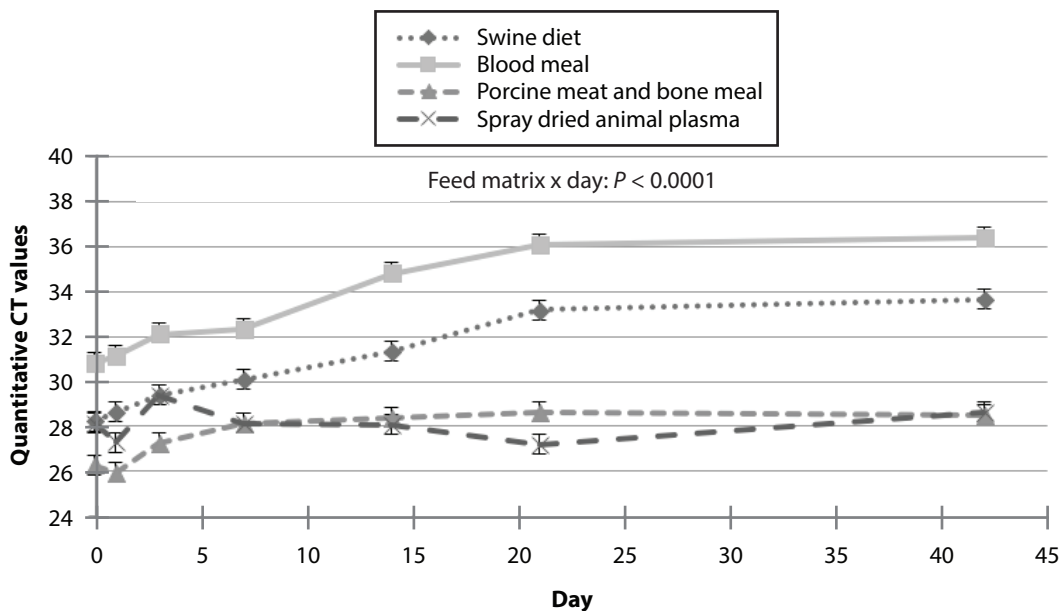


Figure 5. Influence of chemical treatment on RT-PCR detection of PEDV in post-treatment PEDV-inoculated untreated controls stored at room temperature
 Data were analyzed by PCR with each data point represented by N=3. The higher the Ct value, the less quantity of PEDV RNA genetic material is detected.