RESEARCH REPORT
SWINE HEALTH

Title: Post-processing contamination chemical mitigation strategies to control PEDv in feed and feed ingredients – **NPB #14-158**

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Industry summary: Post–processing contamination of PEDv in feed and feed ingredients is a significant concern to the swine industry. Irradiation and thermal processing have both been hypothesized as possible mitigants of the virus, but both are point-in-time solutions that do not provide residual benefits to prevent potential recontamination or cross-contamination within manufacturing, transportation, or storage. This study aimed to find a possible mitigation strategy to help minimize the threat of recontamination in feed and feed ingredients. The results suggested that feed and/or feed ingredients can be treated with different chemical treatments as a means to mitigate PEDv contamination, with medium chain fatty acids, essential oils, and formaldehyde being particularly effective. Importantly, the success of various chemical mitigants was dependent upon matrix, and the PEDv stability over time was also matrix-dependent, and more stable in meat and bone meal and spray-dried animal plasma compared to blood meal or a complete swine diet. This research helps provide potential mitigation solutions that can mitigate PEDv infectivity when transmitted by feed, and thereby ultimately lessen PEDv associated losses to the swine industry.

Keywords: PEDv, chemical mitigation, feed matrix, swine

Scientific abstract: The objective of this experiment was to evaluate the effectiveness of various chemicals to mitigate PEDv in swine feed and ingredients. Treatments were arranged in a 7 × 4 factorial with 7 chemical treatments and 4 feed matrices. The chemical treatments included: 1) negative control with no chemical addition, 2) 0.003% commercial formaldehyde, 3) 1% sodium bisulfate, 4) 1% sodium chlorate, 5) 3% organic acid blend 6) 2% essential oil blend, and 7) 2% medium chain fatty acid blend. The 4 matrices included: 1) complete swine diet, 2) blood meal, 3) meat and bone meal, and 4) spray-dried animal plasma. Matrices were first chemically treated, then inoculated with 5.6 x 10^4 TCID50/g PEDv, stored at room temperature, and

analyzed by real-time PCR on d 0, 1, 3, 7, 14, 21, and 42. The analyzed values represent threshold cycle (CT) at which the virus was detected, and thus lower values indicate greater nucleic acid presence, not infectivity. Commercial formaldehyde, medium chain fatty acid, essential oil and organic acid addition each decreased RNA concentration of PEDv compared to the control ($P < 0.05$), with the commercial formaldehyde treatment being the most effective on d 0 by decreasing the CT by 1.4 to 2.8 CT compared to the control. Feed matrix appears important in retention of PEDv as RNA concentrations were 1.2 to 3.8 CT higher in the complete 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 only improved 0.7 and 2.9 CT by d 42 for spray-dried animal plasma and meat and bone meal, respectively, compared to 4.1 and 5.6 CT for the complete swine diet and blood meal. In summary, time, commercial formaldehyde, and organic acid treatments all enhance the RNA degradation of PEDv in swine feed and ingredients, but their effectiveness varies within matrix. More research is needed to relate RNA concentration to infectivity and to elucidate the appropriate chemical concentration for each feed ingredient or diet.

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 [1, 2]. The coronavirus affects pigs of all life stages, but the highest mortality rates are seen within sucking pigs because of their less developed digestive tracts [3]. 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 [1, 3]. Viral transmission may be by direct contamination, but is more likely from cross-contamination during the manufacturing, transportation, and storage of feed and ingredients [4].Viral destruction by thermal processing or even irradiation is 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. Chemical additives, such as formaldehyde have been demonstrated as effective at mitigating *Salmonella* in animal feed, and research suggests it may be effective in PEDv destruction, as well [5, 6]. However, formaldehyde addition is only approved for *Salmonella* mitigation in the United States (not PEDv), requires specialized equipment for successful application, carries potential worker health concerns, and may be perceived negatively by public consumers [7, 8]. Alternative chemical additives have been additives may provide additional solutions. Medium chain fatty acids, have shown to be effective against enveloped viruses and bacteria, but the concentrations that are required to inactivate the virus can be upward of 20-fold the normal addition levels [9, 10]. Organic acids have long been studied as an antimicrobial agent for several decades, and have shown effective at bacterial mitigation [10, 11]. Organic acids have also been demonstrated to be effective against bacteria and some extremely detrimental viruses, including foot and mouth disease and African swine fever [12]. Essential oils have also showed antimicrobial effects, as well as antiviral RNA effects [13]. Sodium bisulfate is a commercial product, which is used in the broiler and pet food industry for microbial control, particularly against *Salmonella*, but its desiccant and acidulant properties warrant evaluation for effectiveness against PEDv, because its dry powder form may be more easily implemented by the feed industry compared to other liquid chemicals [14]. Finally, sodium chlorate has shown to be effective at pathogen mitigation when included in drinking water of livestock [15] and at PEDv mitigation of surfaces [16]. Because of various physical states, chemical composition, and electrostatic properties of each chemical additive and feed matrix, each additive may interact differently as a mitigant. Therefore, the objective of this experiment was to evaluate the effectiveness of various chemicals to mitigate post-processing PEDv contamination in swine feed and feed ingredients.

Objective: Evaluate the effectiveness of various chemicals to mitigate PEDv in swine feed and ingredients as measured by RT-PCR.

Materials and Methods:

Chemical treatment. Seven chemical treatments were applied to four different feed matrices. The chemical treatments included: 1) negative control with no chemical addition, 2) 0.003% commercial formaldehyde product (Termin-8, Antiox Corp, Lawrenceville, GA), 3) 1% sodium bisulfate (Jones-Hamilton Co, Walbridge, OH), 4) 1% sodium chlorate, 5) 3% organic acid 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 4 matrices included: 1) complete swine diet, 2) blood meal, 3) porcine meat and bone meal, and 4) spray-dried porcine plasma. None of the matrices had previous chemical mitigants added. Each of the feed matrices were tested PEDv negative by RT-PCR prior to the chemical treatments. One kilogram (kg) of each feed matrices was placed in a lab scale ribbon mixer where the liquid chemicals were fogged onto the feed and the powered treatments were mixed directly into the mixer. All chemical treatments were applied on a wt/wt basis. The dry powder treatments were mixed for 3 minutes, the essential oil treatment mixed for 15 minutes because of the known viscosity of the product, and all other liquid treatments were mixed for 5 minutes. Once the treatments were mixed, a total of 90 g 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 remove all organic residue. Between different chemical treatments, the mixer was physically and wet cleaned and dried to remove all organic and chemical residue. 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 Veterinarian Diagnostic Lab with 5.6 x 104 TCID 50 per g of feed. A total of 10 mL (1 ml cell fluid + 9 ml cell culture fluid) was added to each 90 g sample to result in 100 g of inoculated feed matrix. The 10 mL inoculum was added by two 5 mL 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 3-g sub-samples and placed into 15 mL conical tubes. Tubes were stored at room temperature until analyzed by RT-PCR. There was 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 or each subsequent day analysis to determine intra- and inter-assay variation. There was very little variation among sampling day or within duplicate, suggesting that the RT-PCR assay was highly sensitive, accurate, and precise (Table 1).

Results (see attached figures and tables):

All main effects and interactions were highly significant ($P < 0.001$; Table 2). Overall, the commercial formaldehyde product, medium chain fatty acids, essential oils, organic acids, 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 medium chain fatty acid (31.4 CT) essential oil (30.5 CT), and organic acid treatments (30.4 CT), all of which improved ($P < 0.05$) the quantity of detectible PEDv nucleic acid compared to the untreated control as detected by RT-PCR (Table 3).

Significant differences were also observed between each of the feed matrixes ($P < 0.05$). 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).

The interactions are shown 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 relative constant (Figure 1). Of the tested chemical mitigants in the complete swine diet, the medium chain fatty acid treatment was the most effective overall, with the essential oil 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 (Figure 2). Although the essential oil 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 mitigative effects, even though differences were statistically significant (Figure 3).

The PEDv CT in the untreated control of the spray-dried porcine plasma was also relatively stable over time (Figure 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 over time according to RT-PCR (Figure 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.

Discussion:

The purpose of this experiment was to evaluate possible chemical treatments as PEDv mitigation strategies by the use of RT-PCR analysis. Surprisingly, the nucleic acid concentration of PEDv, as measured by RT-PCR, was relatively stable within spray-dried porcine plasma and porcine meat and bone meal, while the nucleic acid of the virus degraded during the initial 21-d period in the complete swine diet and blood meal. Under the laboratory conditions, PEDv was successfully mitigated in different feed matrices by commercial formaldehyde product, medium chain fatty acids, organic acids, and even essential oils to some extent. Interestingly, the most effect mitigants were liquids compared to dry powders, suggesting that physical properties of chemical mitigants are important to consider. In this study, both the sodium bisulfate and sodium chlorate powder products were in a granular form that were larger than some of the feed particles. This may explain their lesser effectiveness in PEDv mitigation within feed and ingredients.

While the objectives of this experiment were reached, it is important to acknowledge that these findings are limited because there was no in vivo or swine bioassay to evaluate infectivity of the virus. The quantity of viral nucleic acid was able to be quantified, which is associated with infectivity, but is not conclusive of infection. Still, this is the first research of its kind to evaluate chemical mitigation of PEDv in swine feed and ingredients, and provides valuable information to control the virus by preventing post-processing cross-contamination. Further research is needed to correlate findings with infectivity, confirm chemical treatment responses, and refine treatments for both effectiveness and applicability to the swine industry.

Conclusions: Time, commercial formaldehyde product, medium chain fatty acids, essential oils, and organic acids all enhance the RNA degradation of PEDv in the tested swine feed and ingredients, but their effectiveness varies within matrix. The viral nucleic acid degrades substantially in blood meal and a complete swine diet by d 21, but is relatively stable in spray-dried animal plasma and porcine meat and bone meal. Further research is needed to correlate findings with infectivity, confirm chemical treatment responses, and refine treatments for both effectiveness and applicability to the swine industry.

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Table 1. Within day laboratory controls to evaluate the inter assay variation[1]

	Day													
Item	0	1		3		7		14		21		42		
Complete 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	
SD Porcine 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	
Average across matrix	28.3	28.3		28.4		28.6		28.1		28.3		28.2		

[1]Values are represented by quantified CT value.

Table 2. Main effects and interaction 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. Treatment main effect for chemical means for chemically treated PEDv inoculated feed matrices[1]

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[e]	32.5[a]	0.08	< 0.0001

[1] A total of 582 samples were used for the analysis.

[2] Cycle 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.

Table 4. Treatment main effects for feed matrix means for chemically treated PEDv inoculated feed matrices[1]

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 582 samples were used for the analysis.

[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. Treatment main effects for day means for chemically treated PEDv inoculated feed matrices[1]

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

[1] A total of 582 samples were used for the analysis.

[2] Cycle 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.

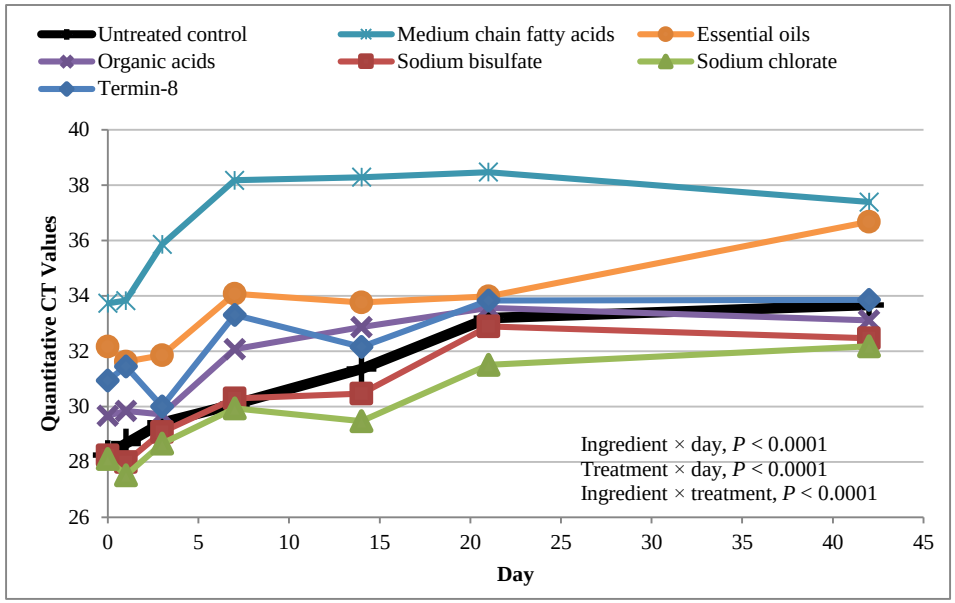

Figure 1. PEDv contamination post-treatment in complete swine diet stored at room temperature. The higher the CT value, the less quantity of PEDv RNA genetic material is detected.

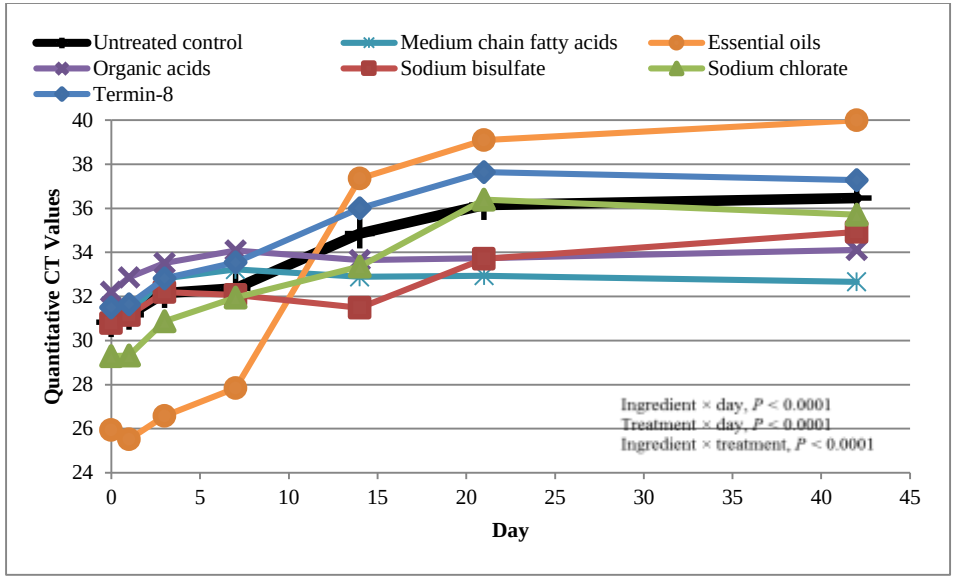

Figure 2. PEDv contamination post-treatment in blood meal stored at room temperature. The higher the CT value, the less quantity of PEDv RNA genetic material is detected**.**

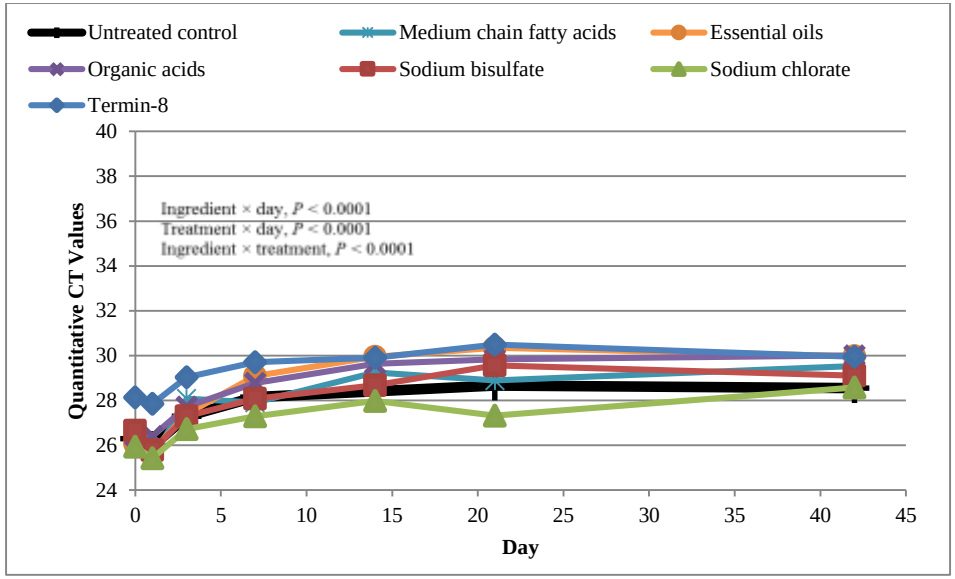

Figure 3. PEDv contamination post-treatment in porcine meat and bone meal stored at room temperature. The higher the CT value, the less quantity of PEDv RNA genetic material is detected.

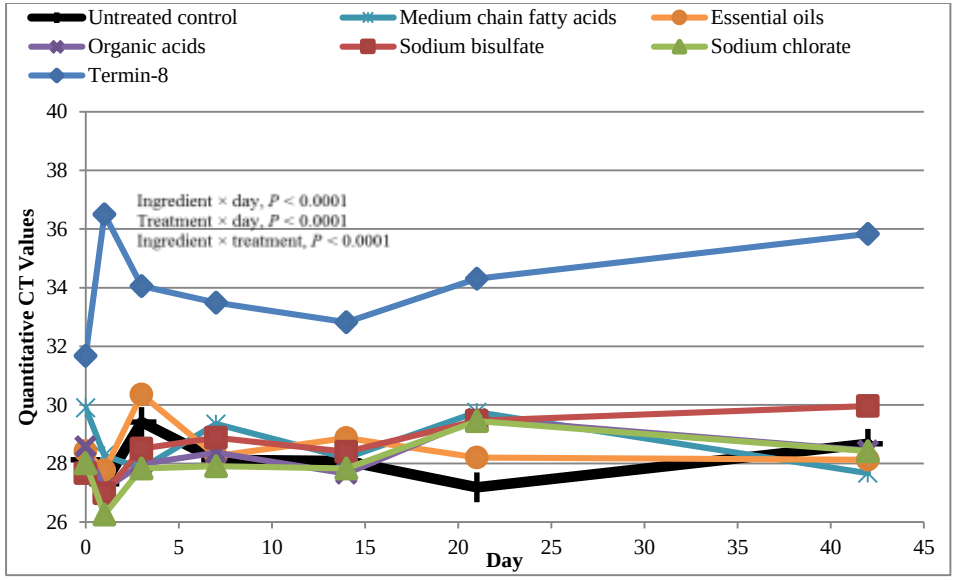

Figure 4. PEDv contamination post-treatment in spray dried animal plasma stored at room temperature. The higher the CT value, the less quantity of PEDv RNA genetic material is detected.

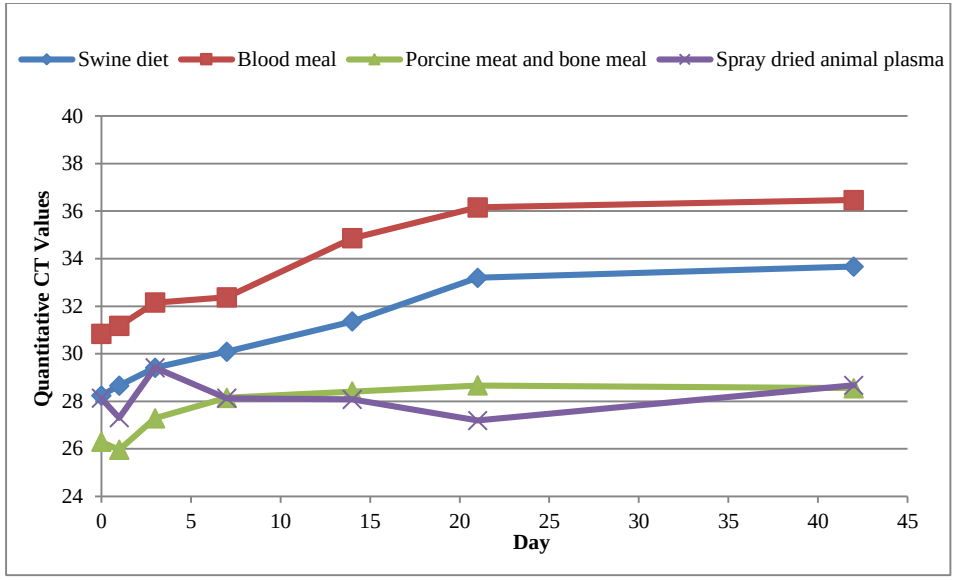

Figure 5. PEDv contamination post-treatment for the untreated controls. The higher the CT value, the less quantity of PEDv RNA genetic material is detected.