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FINAL RESEARCH GRANT REPORT FORMAT

Project Title and Project identification number: Efficacy of chemical mitigants in reducing the risk of pathogen transmission via contaminated feed (SHIC 18-137)

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Industry Summary:

Feed biosecurity became a topic of much interest to the swine interest, given recent results suggesting that feed can harbor viable viral pathogens and potentially serve as source of infection to susceptible pigs. The goal of this study was to evaluate the mitigation potential of chemical feed additives following natural consumption of contaminated and mitigated feed. To determine whether chemical mitigation of feed could reduce or prevent pathogen transmission through feed, we performed a feed trial experiment in which animals were allowed to ingest contaminated or contaminated and mitigated feed for 3 consecutive days. After feeding, each animal was sampled individually and levels of viremia, virus shedding and viral load in tissues were determined by RT-qPCR. For this *in vivo* trial we selected 3 candidate mitigants A, B and C from our previous *in vitro* mitigation project. Results of our trial show that only mitigant A reduced SVA transmission through feed. Whereas no significant differences between control non-mitigated and mitigated feed were observed for PEDV. Results here under conditions in which each animal ingested contaminated and mitigated feed, show that that chemical mitigation alone (with mitigants A, B and C) may not be able to prevent transmission of pathogens through feed. These findings can be likely attributed to many factors, including: **1)** the dose of virus used in our trial; **2)** the fact that the chemical mitigants don't reduce viral load in feed to levels that are non-infectious to susceptible pigs; and **3)** poor contact time of the mitigant with the virus. Therefore alternative strategies such as storage time and importation of feed ingredients from known and trusted sources should also be carefully considered to safeguard the US swine industry from unwanted viral pathogens that are endemic in other regions of the world. Additionally, studies on the mechanism of action of potential mitigants may also allow selection of those compounds that present the greatest chance of virus inactivation in the feed matrix.

Keywords: feed biosecurity, mitigation, feed additives.

Scientific Abstract:

Feed biosecurity became a topic of much interest to the swine interest, given recent results suggesting that feed can harbor viable viral pathogens and potentially serve as source of infection to susceptible pigs. The goal of this study was to evaluate the mitigation potential of chemical feed additives following natural consumption of contaminated and mitigated feed. To determine whether chemical mitigation of feed could reduce or prevent pathogen transmission through feed, we performed a feed trial experiment in which animals were allowed to ingest contaminated or contaminated and mitigated feed for 3 consecutive days. After feeding, each animal was sampled individually and levels of viremia, virus shedding and viral load in tissues were determined by RT-qPCR. For this *in vivo* trial we selected 3 candidate mitigants A, B and C from our previous *in vitro* mitigation project. Results of our trial show that only mitigant A reduced SVA transmission through feed. Whereas no significant differences between control non-mitigated and mitigated feed were observed for PEDV. Results here under conditions in which each animal ingested contaminated and mitigated feed, show that that chemical mitigation alone (with mitigants A, B and C) may not be able to prevent transmission of

pathogens through feed. These findings can be likely attributed to many factors, including: **1)** the dose of virus used in our trial; **2)** the fact that the chemical mitigants don't reduce viral load in feed to levels that are non-infectious to susceptible pigs; and **3)** poor contact time of the mitigant with the virus. Therefore alternative strategies such as storage time and importation of feed ingredients from known and trusted sources should also be carefully considered to safeguard the US swine industry from unwanted viral pathogens that are endemic in other regions of the world. Additionally, studies on the mechanism of action of potential mitigants may also allow selection of those compounds that present the greatest chance of virus inactivation in the feed matrix.

Introduction:

The North American swine industry is under constant threat of foreign animal disease (FAD) entry. Should a pathogen such as Foot and Mouth Disease Virus (FMDV) ever infect the US swine population via accidental or intentional introduction, it would cripple the country's export markets and cause significant animal suffering and economic loss. In recent years, the introduction of two strains of PEDV and Porcine Delta-Coronavirus to the US has exposed significant gaps in our ability to biosecure our livestock populations. While the root cause of pathogen entry has not been determined, published data suggest that contaminated feed ingredients may have played a role (1). To better understand this risk, a model simulating the shipping of feed ingredients or other animal feed products from China to the US was used to identify "high risk" combinations of viruses and feed ingredients (2). Results from this study show that several important swine viral pathogens or surrogates of swine pathogens survive in different feed matrices or feed products that are imported into the US to be used in livestock ration formulations (2). Among the viruses that survived the simulated transportation conditions in our study are Senecavirus A (SVA; surrogate for foot-and-mouth-disease virus [FMDV]), feline calicivirus (FCV; surrogate for vesicular exanthema of swine [VESV]), bovine herpesvirus type 1 (BoHV-1 surrogate for pseudorabies virus [PRV]), porcine reproductive and respiratory syndrome virus (PRRSV), porcine sapelovirus (PSV) (surrogate for swine vesicular disease virus [SVDV]), African swine fever virus (ASFV) and porcine circovirus 2 (PCV2) (2). These results provided firsthand evidence suggesting that contaminated feed ingredients may pose a risk for transboundary movement of swine pathogens and are likely to play a role on pathogen transmission at global and/or national level.

These observations raised an important question 1) Are there any feed additives that can be used to mitigate the risk of pathogen transmission through contaminated feed? A previous study conducted by our group and supported by the SHIC (Project 17-187), identified candidate feed additives capable of decreasing viral load in feed as determined by virus isolation and viral quantitation in cell culture *in vitro*. The goal of the present study was to expand on this work and to assess whether addition of these feed additives to contaminated feed can prevent infection of pigs after natural ingestion of contaminated and treated feed. The overall goal of the proposed study is to assess the efficacy of select feed additives (including two medium chain fatty acid blends and a blend of organic acids) in reducing the risk of transmission of PEDV, PRRSV and SVA following ingestion of contaminated and treated feed.

Objectives:

The overall goal of this study is to identify feed additives that could potentially be used as mitigants to reduce the risk of pathogen transmission through contaminated feed. Specific aim of the study was:

The objective of the proposed study is:

Objective 1: *To assess the efficacy of chemical mitigants to prevent infection of pigs after ingestion of contaminated feed.* The specific aims related to this objective are:

Specific aim 1: To determine the efficacy of feed additives (top three from current project) in reducing the risk of transmission of SVA, PRRSV and PEDV through contaminated feed; and

Specific aim 2: To determine the efficacy of the selected mitigants (top three from current project) against different contamination levels of the target pathogens.

Materials & Methods:

Cells and viruses. Appropriate cell cultures susceptible to each of the target viruses (SVA, PEDV and PRRSV) were used to amplify and prepare viral stocks to spike each feed ingredient. SVA strain SD15-26 (4) was amplified in H1299 cells (ATCC® CRL-5803™). PRRSV virus strain SD1-7-4 was amplified in MARC145 cells, while PEDV was amplified in Vero cells (ATCC® CRL-1586™). H1299 cells were maintained in RPMI medium (Corning; ThermoFisher) while Vero and MARC145 cells were maintained in minimal essential medium (MEM). Both RPMI and MEM media were supplemented with 10% fetal bovine serum (FBS), 2 mM of L-glutamine (Corning), 100 U/ml of penicillin (Gibco), 100 µg/mL of streptomycin (Gibco), 50 µg/mL gentamicin sulfate (VWR), 2.5 µg/mL of amphotericin B (Corning). For virus propagation, 75-cm² flasks containing 70 to 85% confluent cell monolayers were inoculated with the appropriate virus and incubated at 37°C for 48 to 120 h depending on the virus strain. Following one freeze-thaw cycle, the suspension was centrifuged for 10 min at 1,000 × g. Cell supernatant was collected, aliquot, and stored at -80°C until use. The virus stocks were titrated in 96-well microtiter plates by endpoint dilution. Titers were calculated and expressed as median TCID₅₀/ml.

Selection of mitigants/feed additives. The top performing feed additives identified in project #17-187 were tested in the natural feed ingestion model here. A list of the mitigants tested, their composition and inclusion rate are presented in Table 1.

Table 1 – Feed additives tested as mitigants against SVA, PRRSV, PEDV and BoHV-1.

Mitigant ID	Composition	Inclusion rate
A	medium chain fatty acids blend of organic acids and methionine hydroxy analogue	1.50%
C	(HMTBa)	0.50%
B (MCFA C8+C10)	medium chain fatty acids blend	1%

Feed formulation and contamination. To assess the efficacy of the selected chemical mitigants (Table 1) against SVA, PRRSV and PEDV complete weaned-piglet feed was formulated by the swine nutrition group and mixed at the SDSU swine unit feed mill. Each feed additive was mixed with the other feed ingredients at the SDSU feed mill. Mitigated feed was prepared to feed the animals for 14 days of the experimental period. Mitigated feed was available to the animals throughout the 14 day experiment.

Feed contamination. Complete feed was be spiked with three doses of each target virus: PEDV (10⁵ [high], 10⁴ [medium] or 10³ [low] TCID₅₀), PRRSV (10⁶ [high], 10⁵ [medium] or 10⁴ [low] TCID₅₀) and SVA (10⁷ [high], 10⁶ [medium] or 10⁵ [low] TCID₅₀). Each viral dose was mixed with 50 g of feed which were then pooled into

200 g samples which were stored at 10-15°C for 3 days prior to being feed to each group of animals (n=4; 50g/animal/day). Contaminated/mitigated feed was fed to all groups for 3 consecutive days. To ensure that all animal in the groups would ingest the feed, we subjected them to a 12-h feed withdrawal prior to feeding the contaminated feed.

Animal trials. After complete feed containing the mitigants in Table 1 was formulated and spiked with the target viruses as described above, the efficacy of the mitigants in neutralizing virus infectivity was assessed in 3-4-week-old piglets following natural feed ingestion. The experimental design of the animal trials is presented in Figure 1. Each animal within the groups shown in Figure 1 was allowed to ingest ~50g of contaminated/treated feed per day for 3 days. This approach allowed us to test the selected mitigants against three doses of virus (high, medium and low).

Animals were monitored daily for clinical signs of disease and serum was collected on days 0, 3, 7, and 14 pi and tested by real-time PCR for the target pathogens. Additionally, rectal swabs were collected from the PEDV-exposed group on days 0, 3, 7 and 14 pi and tested by real-time PCR. Serum samples collected on day 14 pi were used to assess seroconversion to the target pathogens (ELISA [PRRSV] or VN assays). All animals were euthanized on experimental day 14 and tissues collected and tested as described above. Results from the qPCR in serum (SVA, PRRSV and PEDV), rectal swabs (PEDV) and tissues (SVA, PRRSV and PEDV) were analyzed in conjunction with serology results to assess infection by each virus.

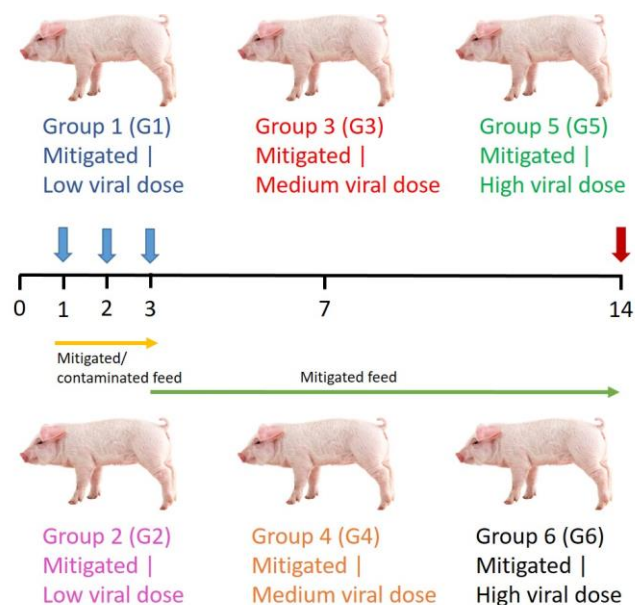


Figure 1 – Animal experimental design of oral mitigation trials. Each group contained 4 animals (n=4). Contaminated and mitigated feed was provided for 3 days (1-3). Mitigated feed was provided for the duration of the 14 day experiment. This experimental design was used for each mitigant (A, B and C) and viral pathogen tested (SVA, PEDV and PRRSV).

Nucleic acid extraction and RT-qPCR. Clinical samples described above were used for nucleic acid extraction using the MagMax 96 viral isolation kit (Life technologies, Waltham MA, USA). A 175- μ l sample was used for the extractions performed on an automated extractor Qiacube

HT (Qiagen). Commercially available real-time PCR kits were used for the detection of SVA (EZ-SVA, Tetracore Inc.), PEDV (EZ-PED/TGE/PDCoV MPX, Tetracore Inc.), and PRRSV (PRRSV EZ-PRRSV MPX 4.0, Tetracore Inc.) in clinical samples collected from the animals in the experiment. Quantitative RT-qPCRs were run for each viral pathogen. For this, standard curves were established by using stock viral suspensions through 10-fold serial dilutions from 10^{-1} to 10^{-8} . Relative viral genome copy numbers were calculated based on the standard curve determined using the four-parameter logistic regression model function within MasterPlex Readerfit 2010 software (Hitachi Software Engineering America, Ltd., San Francisco, CA). The amount of viral RNA detected in samples were expressed as \log_{10} (genome copy number)/mL.

Serological assays. Serum samples collected on days 0 and 14 were subjected to serological assays. Neutralization assays for SVA and PEDV were performed as previously described (Joshi et al., 2016; Hain et al., 2016). PRRSV ELISAs were performed at the Animal Disease Research Diagnostic Laboratory (ADRDL).

Results

Assessing the efficacy of Mitigant A (medium chain fatty acid) blend against target viruses.

To assess whether addition of chemical mitigants to feed ingredients was effective in neutralizing virus infectivity, all clinical samples collected from animals exposed to feed contaminated with SVA, PEDV and PRRSV and mitigated with Mitigant A, were subjected to RT-qPCR examination. Following ingestion of virus contaminated/mitigated feed (treatment groups) or virus contaminated non-mitigated feed (positive control groups) clinical samples were collected at different intervals and virus infection was assessed by RT-qPCR and serological assays.

- **Mitigant A in SVA spiked feed.** The efficacy of mitigant A in reducing SVA infection through the oral route was monitored against three doses of SVA (10^5 , 10^6 and 10^7 TCID₅₀). After consumption of contaminated and mitigated feed, we assessed levels of viremia, virus shedding and viral load in the tonsil by RT-qPCR. As shown in **Figure 2A**, no differences in the viremia levels were observed in animals that received mitigated or non-mitigated feed in any of the viral contamination doses tested in our experiment. Interestingly, when animals were exposed to 10^5 TCID₅₀ of SVA, addition of mitigant A to the feed resulted in lower virus shedding in feces (**Figure 2B**; * $p < 0.05$) and lower viral load in the tonsil (**Figure 2C**; * $p < 0.05$). When SVA contamination levels were increased to 10^6 or 10^7 TCID₅₀ no differences in virus shedding nor viral load in tonsil were observed between animals receiving mitigated and non-mitigated feed.

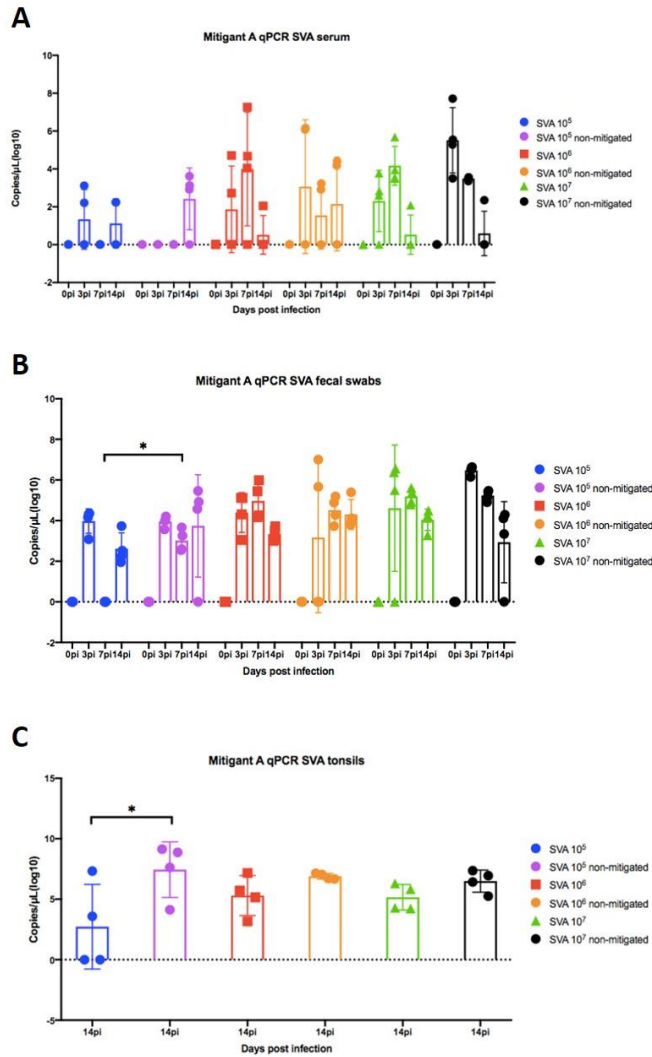


Figure 2 – Effect of mitigant A on oral infection by Senecavirus A. **(A)** Viremia levels in serum collected on days 0, 3, 7 and 14 pi as determined by RT-qPCR. **(B)** Viral shedding detected in rectal swabs by RT-qPCR. **(C)** Viral load in tonsil detected on day 14 pi by RT-qPCR.

Virus neutralization titers detected on day 14 pi, are consistent with viral findings (virus shedding and viral load) suggesting a modest effect of mitigant A on SVA. As shown in **Figure 3A**, lower levels of neutralizing antibodies were detected in animals that were exposed to the low SVA dose (* $p < 0.05$; 10⁵ TCID₅₀). No effects of the mitigation were observed, however, when the animals were exposed to higher oral doses of SVA (10⁶ and 10⁷ TCID₅₀).

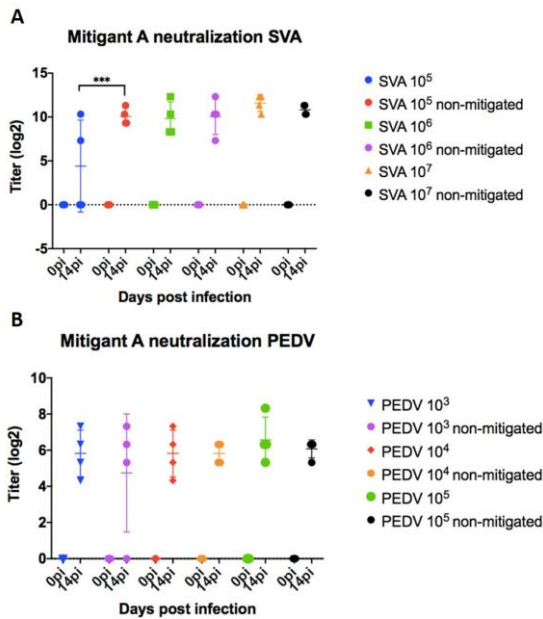


Figure 3 – Virus neutralization assays for SVA (A) and PEDV (B) after mitigation of feed with mitigant A.

- Mitigant A in PEDV spiked feed.** The efficacy of mitigant A in reducing PEDV infection through the oral route was monitored against three doses of the virus (10³, 10⁴ and 10⁵ TCID₅₀). After consumption of contaminated and mitigated feed, we assessed virus shedding in feces and viral load in the small intestine by RT-qPCR. As shown in **Figure 4A and B**, although a decreased trend in virus shedding and viral load was observed between mitigated and non-mitigated treatment groups, no significant differences were noted. Additionally, virus neutralization assays confirmed that all groups were infected with PEDV, as evidenced by seroconversion on day 14 pi (**Figure 3B**).

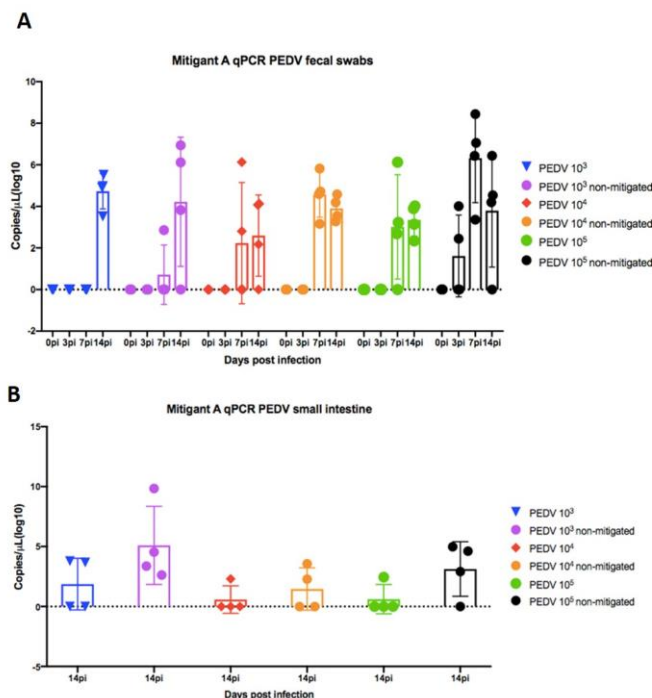


Figure 4 – Effect of mitigant A on oral infection by PEDV. (A) Viral shedding detected in rectal swabs by RT-qPCR. (C) Viral load in small intestine detected on day 14 pi by RT-qPCR.

Assessing the efficacy of Mitigant B against target viruses. To assess whether addition of mitigant B (blend of organic acids and methionine hydroxy analogue) to feed ingredients was effective in neutralizing virus infectivity, all clinical samples collected from animals exposed

to feed contaminated with SVA and PEDV, were subjected to RT-qPCR examination. Following ingestion of virus contaminated/mitigated feed (treatment groups) or virus contaminated non-mitigated feed (positive control groups) clinical samples were collected at different intervals and virus infection was assessed by RT-qPCR and serological assays.

• **Mitigant B in SVA spiked feed.** The efficacy of mitigant B in reducing SVA infection through the oral route was monitored against three doses of SVA (10^5 , 10^6 and 10^7 TCID₅₀). After consumption of contaminated and mitigated feed, we assessed levels of viremia, virus shedding and viral load in the tonsil by RT-qPCR. As shown in **Figure 5A**, no differences in the viremia levels were observed in animals that received mitigated or non-mitigated feed in any of the viral contamination doses tested in our experiment. Similarly, SVA shedding and load in tonsil were similar between animals in mitigated and non-mitigated feed (**Figure 5B and C**). Virus neutralization assays (**Figure 6A**) were consistent with the virological findings and no differences in seroconversion were observed between treated and non-treated groups.

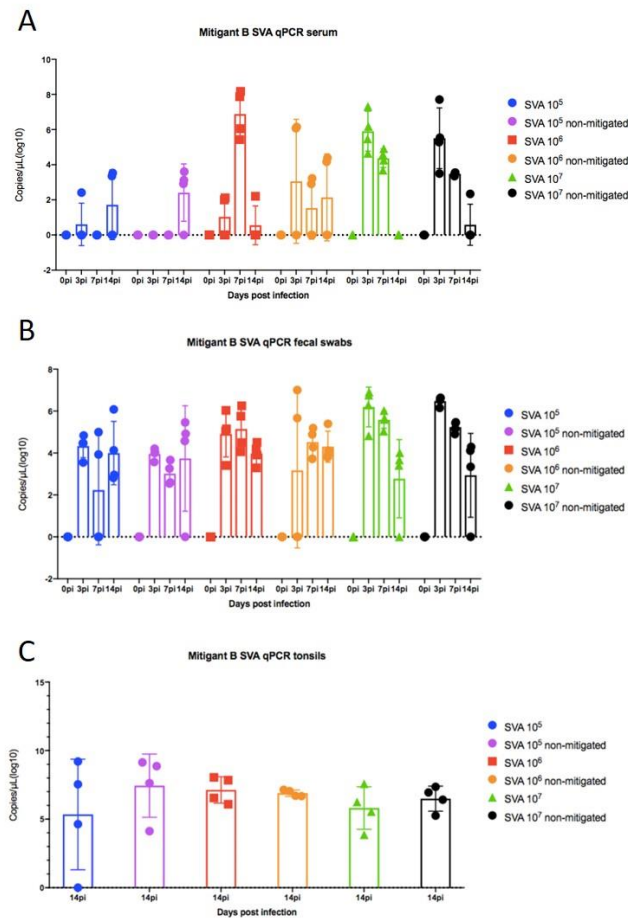


Figure 5 – Effect of mitigant B on oral infection by Senecavirus A. **(A)** Viremia levels in serum collected on days 0, 3, 7 and 14 pi as determined by RT-qPCR. **(B)** Viral shedding detected in rectal swabs by RT-qPCR. **(C)** Viral load in tonsil detected on day 14 pi by RT-qPCR.

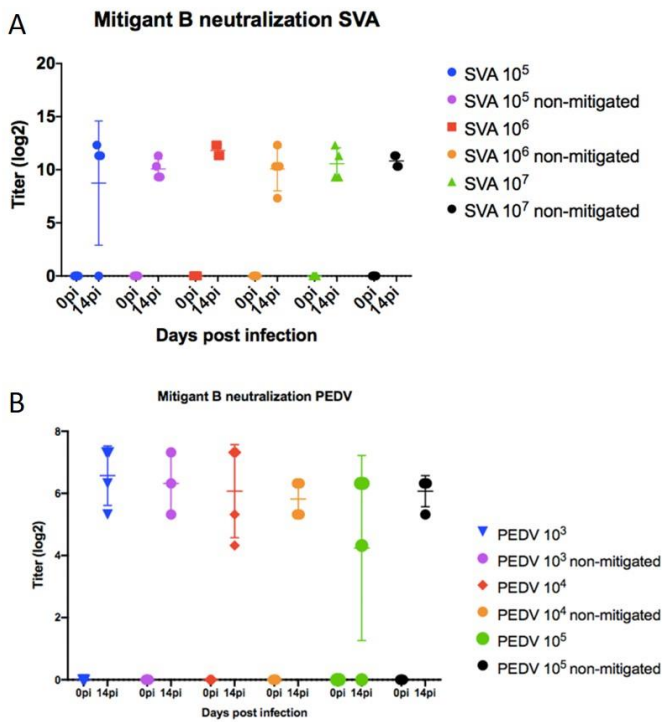


Figure 6 – Virus neutralization assays for SVA (A) and PEDV (B) after mitigation of feed with mitigant A.

- Mitigant B in PEDV spiked feed.** The efficacy of mitigant B in reducing PEDV infection through the oral route was monitored against three doses of the virus (10³, 10⁴ and 10⁵ TCID₅₀). After consumption of contaminated and mitigated feed, we assessed virus shedding in feces and viral load in the small intestine by RT-qPCR. As shown in **Figure 7A and B**, decreased virus shedding was observed between mitigated and non-mitigated treatment groups when the low PEDV contamination dose was used (10³). No differences were noted at the higher viral contamination doses (**Figure 7A and B**). Virus neutralization assays confirmed that all animals were infected by oral exposure to feed contaminated with PEDV, as evidenced by seroconversion on day 14 pi (**Figure 8B**).

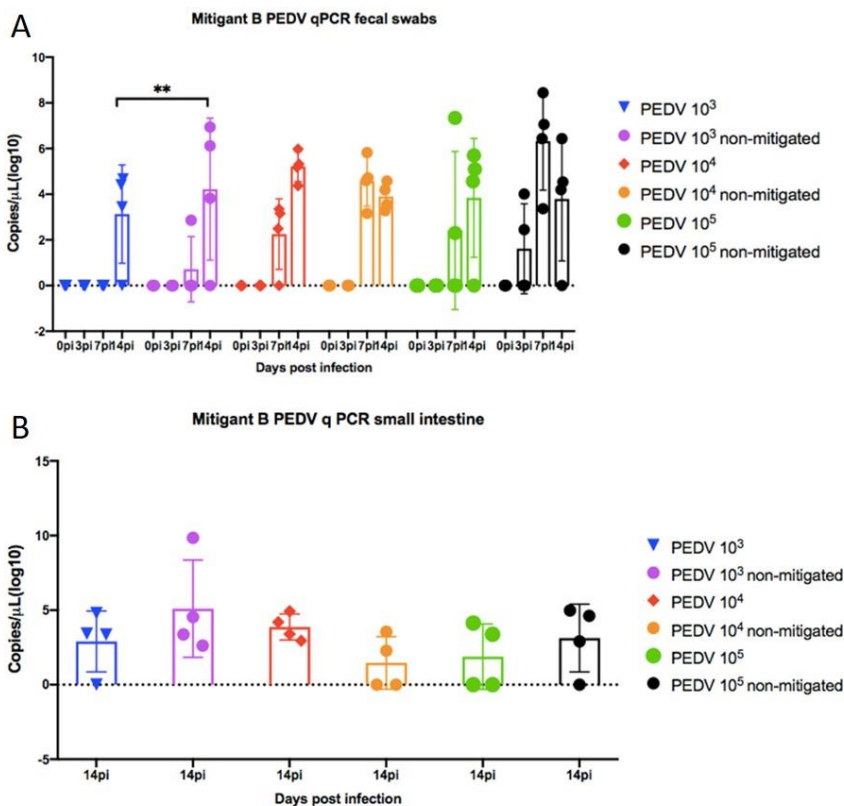


Figure 7 – Effect of mitigant A on oral infection by PEDV. (A) Viral shedding detected in rectal swabs by RT-qPCR. (C) Viral load in small intestine detected on day 14 pi by RT-qPCR.

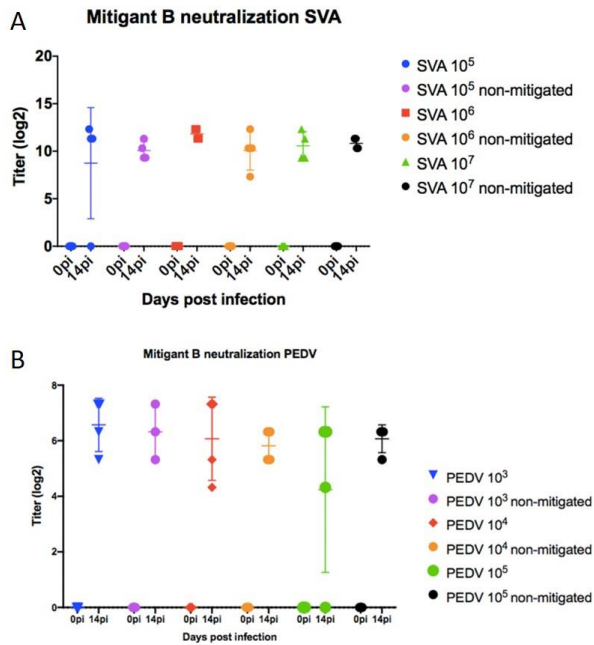


Figure 8 – Virus neutralization assays for SVA (A) and PEDV (B) after mitigation of feed with mitigant B.

Assessing the efficacy of Mitigant C against target viruses. To assess whether addition of mitigant C (blend of medium chain fatty acids) to feed ingredients was effective in neutralizing virus infectivity, all clinical samples collected from animals exposed to feed contaminated with SVA and PEDV, were subjected to RT-qPCR examination. Following ingestion of virus contaminated/mitigated feed (treatment groups) or virus contaminated non-mitigated feed (positive control groups) clinical samples were collected at different intervals and virus infection was assessed by RT-qPCR and serological assays.

Mitigant C in SVA spiked feed. The efficacy of mitigant C in reducing SVA infection through the oral route was monitored against three doses of SVA (10⁵, 10⁶ and 10⁷ TCID₅₀). After consumption of contaminated and mitigated feed, we assessed levels of viremia, virus shedding and viral load in the tonsil by RT-qPCR. As shown in **Figure 9A**, no differences in the viremia levels were observed in animals that received mitigated or non-mitigated feed in any of the viral contamination doses tested in our experiment. Similarly, SVA shedding and load in tonsil were similar between animals in mitigated and non-mitigated feed (**Figure 9B and C**). Virus neutralization assays (**Figure 10A**) were consistent with the virological findings and no differences in seroconversion were observed between treated and non-treated groups.

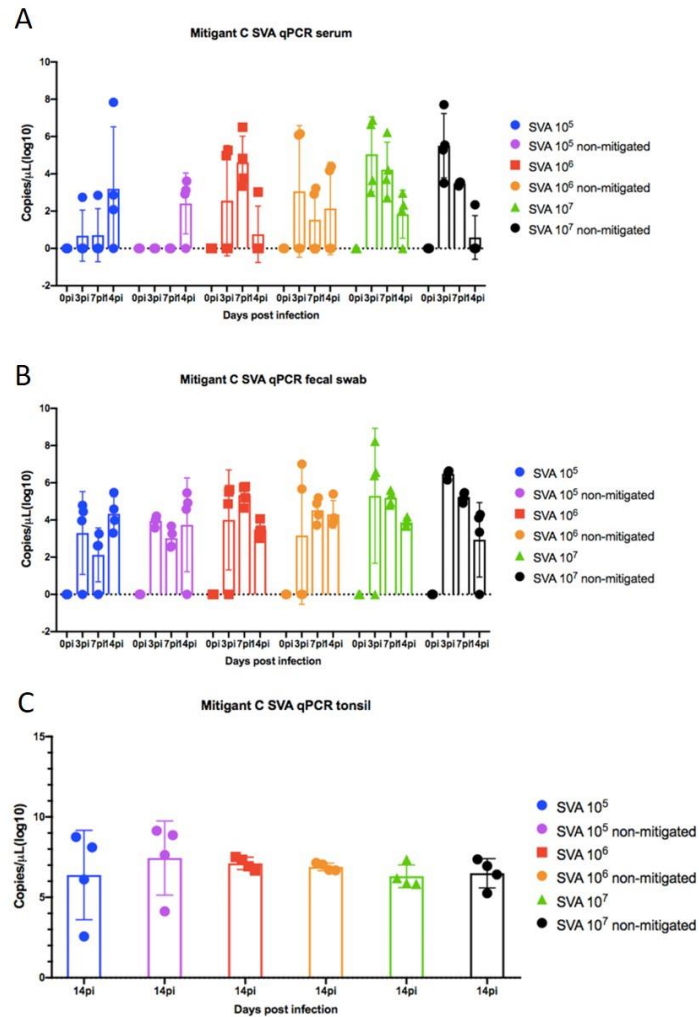


Figure 9 – Effect of mitigant C on oral infection by Senecavirus A. (A) Viremia levels in serum collected on days 0, 3, 7 and 14 pi as determined by RT-qPCR. (B) Viral shedding detected in rectal swabs by RT-qPCR. (C) Viral load in tonsil detected on day 14 pi by RT-qPCR.

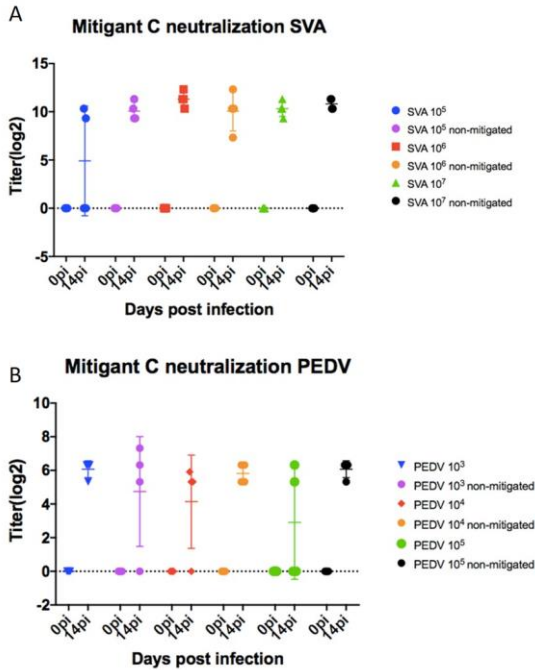


Figure 10 – Virus neutralization assays for SVA (A) and PEDV (B) after mitigation of feed with mitigant C.

• **Mitigant C in PEDV spiked feed.** The efficacy of mitigant C in reducing PEDV infection through the oral route was monitored against three doses of the virus (10^3 , 10^4 and 10^5 TCID₅₀). After consumption of contaminated and mitigated feed, we assessed virus shedding in feces and viral load in the small intestine by RT-qPCR. As shown in **Figure 11A and B**, decreased virus shedding was observed between mitigated and non-mitigated treatment groups when the low PEDV contamination dose was used (10^3). No differences were noted at the higher viral contamination doses (**Figure 11A and B**). Virus neutralization assays confirmed that all animals were infected by oral exposure to feed contaminated with PEDV, as evidenced by seroconversion on day 14 pi (**Figure 10B**).

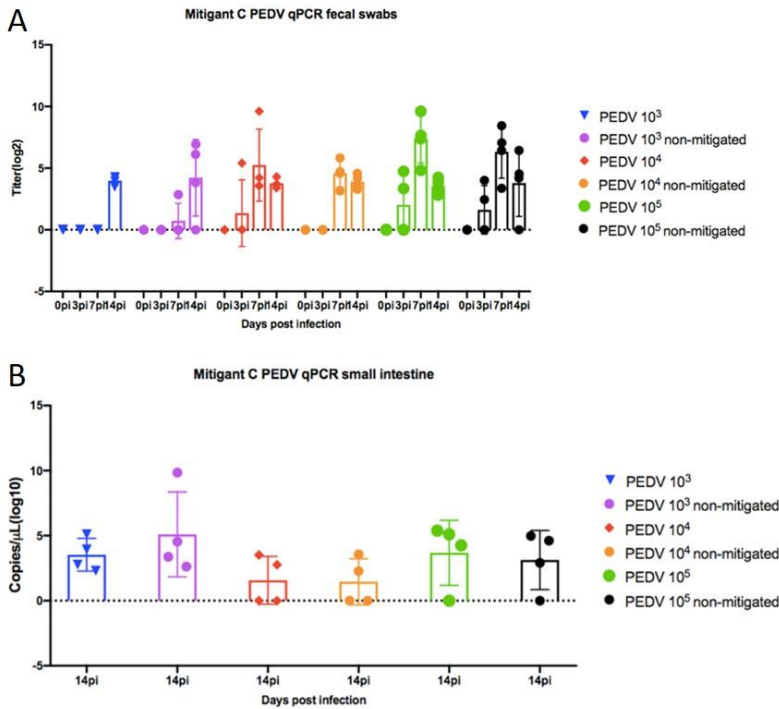


Figure 11 – Effect of mitigant A on oral infection by PEDV. (A) Viral shedding detected in rectal swabs by RT-qPCR. (B) Viral load in small intestine detected on day 14 pi by RT-qPCR.

Assessing the efficacy of Mitigants A, B and C against PRRSV. The effects of mitigant A, B and C were also assessed against PRRSV. However, we did not observe PRRSV infection in animals that received control non-mitigated feed. All clinical samples collected from these animals were negative upon RT-qPCR testing and no seroconversion was detected on day 14 pi. These results precluded us from conducting any analysis on the efficacy of the mitigants against PRRSV.

Discussion:

In this study we assessed the efficacy of chemical feed additives in neutralizing the infectivity of SVA and PEDV through feed. The top three feed additives presenting the highest viral neutralizing activity *in vitro* (SHIC project #17-187) were tested against different feed contamination levels of SVA, PEDV and PRRSV. The present study was designed to ensure that all animals included in the treatment groups were exposed and ingested contaminated and mitigated feed. Additionally, since the contamination levels of feed in the field are unknown we tested the mitigants against three different doses of each virus (low, medium and high).

During the *in vitro* phase of our project (SHIC project #17-187) feed additives were screened against several swine pathogens. Results from that study showed that a select group of feed additives have the potential to be used as chemical mitigants to decrease the risk of pathogen transmission through feed. Several limitations of the *in vitro* study, however, included the fact that: **1)** mitigation results were based mostly on *in vitro* assays; **2)** a single contamination dose was used (10^5 TCID₅₀) for each pathogen; **3)** it is not known if the reduction in titers observed after mitigation would be sufficient to prevent infection/transmission of those pathogens after ingestion of feed. In the present study, we addressed these limitations by screening the top three mitigants against SVA, PEDV and PRRSV following natural consumption of contaminated and contaminated and mitigated feed.

Results of our study showed that under the conditions used in the animal clinical trial, in which every animal in the study ingested contaminated feed via natural feeding the efficacy of the mitigants was low. Out of the three mitigants tested, only mitigant A effectively neutralized SVA when a low contamination dose of the virus was used (Figure 2A, 2B and 2C) as evidenced by lower levels of virus shedding and viral load in tonsil. Serological data also confirmed an overall lower infectivity of animals that received feed contaminated (10^5 TCID₅₀) and mitigated with Mitigant A. Interestingly, when the contamination levels were increased to 10^6 and 10^7 TCID₅₀, no significant differences were observed between the mitigated and non-mitigated feed, with all animals presenting similar levels of virus shedding, and viral load in tonsil. Surprisingly, no neutralization or mitigation effect were observed against SVA with mitigants B and C. Similarly no mitigation effect was observed against PEDV.

The findings are likely explained by the fact that our previous *in vitro* study showed that addition of these feed additives to porcine feed does not completely neutralize virus infectivity. Results here, further suggest that although these products are capable of lowering the viral titers in feed, the reductions are not sufficient to prevent infection, especially when higher viral contamination levels are initially present in the feed. One important aspect of the experimental design of our study that needs to be kept in mind is the incubation period of the contaminated feed with the mitigant, which was of 3 days. This incubation period was chosen as an appropriate time frame that would be feasible to retain/store mitigated feed prior to feeding it to animals.

Oral infection dose studies have shown that the infectious titers required to infect pigs with ASFV through feed are 100-1000 greater than those needed for the virus to establish infection when given in a liquid media. Notably, contamination of feed with PRRSV (10^4 , 10^5 and 10^6 TCID₅₀) did not result in infection, as no viremia nor virus shedding were detected in animals exposed to the virus. Additionally, lack of infection through the natural feed consumption was

also confirmed by a PRRSV ELISA performed at the SDSU ADRDL. These results suggest that higher titers may be required to successfully establish PRRSV infection through ingestion of contaminated feed. It is important to note, that in our previous virus survival studies (Dee et al., 2018) or *in vitro* mitigation studies, we were not able to isolate PRRSV in cell culture. Viable virus was only detected after intramuscular injection of the supernatant feed ingredients processed in PBS, which resulted in re-constitution of the virus in a liquid interphase.

Results here under conditions in which each animal ingested contaminated and mitigated feed, show that that chemical mitigation of feed alone may not be able to prevent transmission of pathogens through feed. These findings can be likely attributed to many factors, including: **1)** the dose of virus used in our trial; **2)** the fact that the chemical mitigants don't reduce viral load in feed to levels that are non-infectious to susceptible pigs; and **3)** poor contact time of the mitigant with the virus. Therefore alternative strategies such as storage time and importation of feed ingredients from known and trusted sources should also be carefully considered to safeguard the US swine industry from unwanted viral pathogens that are endemic in other regions of the world. Additionally, studies on the mechanism of action of potential mitigants may also allow selection of those compounds that present the greatest chance of virus inactivation in the feed matrix.

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Revised 12/15