Potential Alternatives to Reduce Porcine Epidemic Diarrhea Virus (PEDV) Contamination in Feed Ingredients

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The spreading of porcine epidemic diarrhea (PEDV) in the US has prompted questions about reducing the contamination of this virus in feed ingredients. Although, PEDV has been diagnosed in Europe and Asian since 1971, there is almost no information describing procedures to properly deactivate PEDV especially in the conditions that are typically used for feed or feed ingredients manufacturing. Therefore, disinfection of PEDV under other conditions and information from TGEV and other coronaviruses are also investigated in this review. One caveat of this review is that no attempt was made to quantify the risk of feed route of transmission relative to other forms of PEDV transmission. We continue to believe that while feed transmission is possible, the magnitude of the risk remains undemonstrated and in likelihood is less than other forms of transmission. However, we acknowledge that multiple routes of PEDV transmission are occurring and further information on feed risk and feed mitigation strategies are needed.

1 Physical disinfection

1.1 Heating

Time and temperature to inactivate PEDV in swine feces was studied using an aluminum tray in an incubator to simulate thermally assisted drying and disinfection (TADD) protocols for transport vehicle decontamination. The combination of 160 °F (71°C) for 10 minutes or 68 °F (20°C) for at least 7 days were capable of inactivating PEDV. Other combinations of time and temperature evaluated (145°F (62.8 °C) for 10 mins, 130°F (54.4 °C) for 10 mins, 100°F (37.8 °C) for 10 mins, 68°F (20 °C) for 24 hrs) were not sufficient to inactivate the virus as measured by bioassay (Thomas et al., 2013). Cell culture adapted PEDV was moderately stable at 122°F (50 °C) but lost infectivity if maintained at a temperature higher than 140°F (60 °C) for 30 minutes (Pospischil et al. 2002). From this limited information of the time versus temperature

Theoretical Time and Temperature Disnfection Curve

$y = -13\ln(x) + 187.31$
$R^2 = 0.9964$

1440 mins (1 d) at 92.8 °F

Temp, °F

Time, min
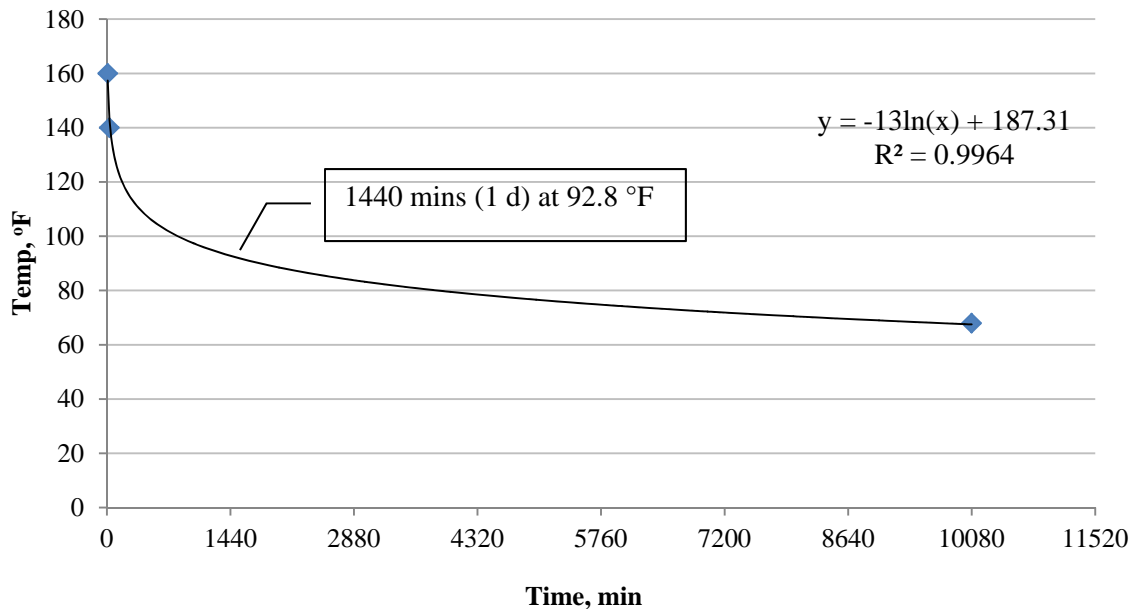
relationship, a graph was created to represent the time and temperature combination to inactivate PED virus.

Due to the limited amount of data used to create this relationship curve, caution should be used for the interpretation of this data. Our objective was to develop a theoretical relationship of time and temperature to deactivate PEDV in thermal processing of feed or feed ingredient. Certainly, if feed or feed ingredients are linked as a major route of transmission this area needs further investigation to quantify the relationship and provide more robust recommendations.

Pelleting is a typical process to provide molding of swine feed mash into pellet that requires moisture, heat, and pressure (Fairfield et al., 2005). In the pelleting process, mash feed is conditioned with liquid and stream in conditioner at temperatures ranging from 160 to 210 °F with a typical retention time of 30 to 60 seconds. Lower temperatures are typically used for nursery pig diets that contain milk and specialty ingredients and higher temperatures are used for higher starch diets like corn soybean meal based diets. Longer retention times can be obtained with specialized conditioners. For example, double pass conditioners can extend retention time to 90 seconds or more. Again, as with time and temperature data, more investigation is needed to provide operational guidelines to provide disinfection efficacy data.

Depending on the desired condition, an expander is installed after the conditioner in some feed mills to provide stream and pressure where conditioning temperature can exceed 250°F (121°C) (Fairfield et al., 2005). The conditioned mash feed then flows to pelleting chamber to form pellet where the temperature is further increased from the friction of feed passing through the die. This rise in temperature can be up to 20 °F. However, it is not well know if this temperature is reached uniformly throughout the pellet. We assume since the rise in temperature is due to friction that the interior of the pellet will have less of a pelleting-induced increase in temperature. Furthermore, if our assumption is correct, then the diameter of the pellet will directly influence the increase in temperature measured at the center of the pellet with larger diameter pellets having lower increases in internal temperature.

Theoretical Time and Temperature Disinfection Curve with Typical Pelleting Conditions shown in the Shaded Area
(Note: This is the same curve as above but with a compressed time scale)
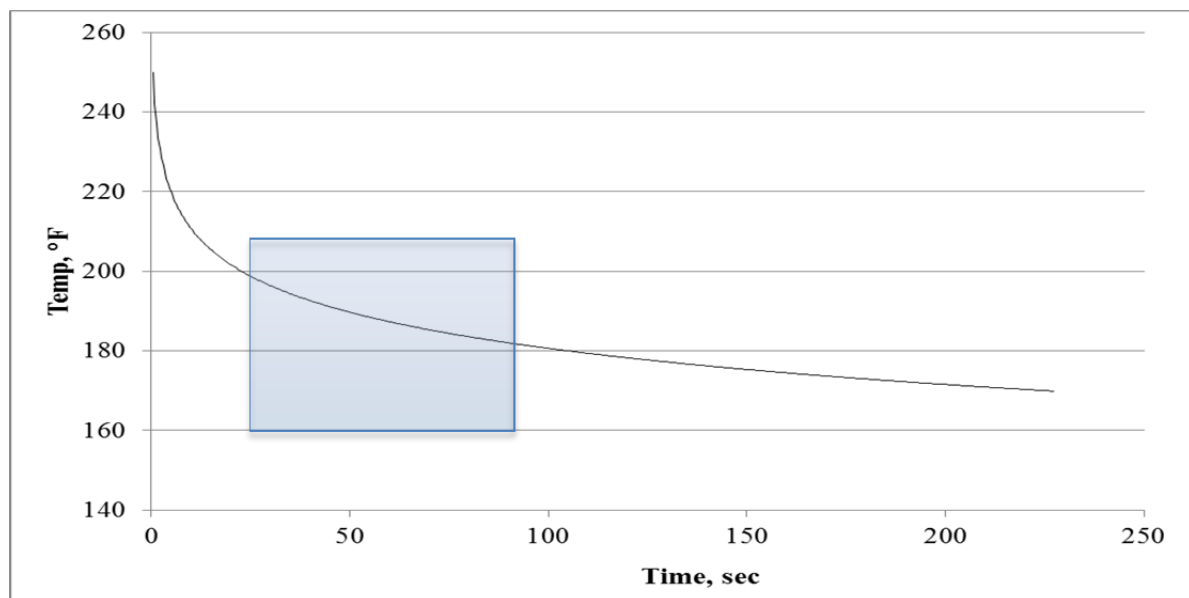
The shaded box in the above graph encompasses typical feed thermal processing temperatures and times. Complex nursery diets with milk and animal products will be processed at the lower end of the temperature range while high grain diets will be processed at the higher end of the temperature range. Utilizing the normal temperature range used to process feed in a typical pelleting system (160 to 210 °F), and considering the theoretical time/temperature

inactivation curve, we are then able to create the theoretical retention time needed to deactivate PEDV in the pelleting process in the following table.

Theoretical Retention Time to Inactivate PEDV considering a Range of Temperature used in Pelleting System

Temperature (°F)	Time (minutes)	Time (seconds)
160	8.2	490
170	3.8	227
180	1.8	105
190	0.8	49
200	0.4	22
210	0.2	10

From the theoretical relationship, it seems that pelleting can help to inactivate the PEDV with the temperature and retention times that are typically used in the conditioner. However, pelleting alone is unlikely to sterilize the feed. The lower range of temperatures (160 to180 °F) may require more retention time than typically used in a conditioner. Thus, further processing with an expander or extended conditioning should facilitate the inactivation of the virus. Unfortunately, expanding nursery diets containing milk products and specialty ingredients has been shown to reduce pig performance. Also, moist heat (stream) as used in pelleting process is known to be more effective and require less time than dry heat (flame, baking) to deactivate viruses (Dvorak, 2008). Therefore, increased moisture in the conditioner may help to shorten the retention time.

This theoretical relationship between temperature and time to inactivate PEDV serves as a guideline to investigate if the temperature and time used in feed ingredient or feed additives manufacturing can inactivate the virus. One important consideration that needs to be kept in mind is that the amount of viral load that is present in the contaminated feed is an important variable as higher temperature and longer time may be needed to reduce such a high viral load.

The temperature between 180 to 200 °F is also used in the process of drying grains with varying length of time depending on quantity of grain, moisture content, and type of grain drier

(Moechnig, 2005). Thus, the grain drying process with proper retention time may help to reduce PEDV contamination of the grain source as well.

1.2 Long term feed storage

We are unaware of any evidence regarding efficacy of long term feed storage and PEDV viability. Limited evidence is provided by laboratory data of moistened feed and dry feed stored at room temperature (Goyal et al., 2013 NPB research updates). For moistened feed, clinical evidence of infection was not noted after initial inoculation. Although, there was PED viral RNA detected for 28 days after inoculation in piglet intestines. For dry feed infectivity or viral RNA was not demonstrated via bioassay past the 1-week time point.

Longer-term storage may be a viable option for bagged feed or ingredients but is going to be impractical for bulk feeds. Based on this data it appears that storage would have to be a minimum of one week at room temperature. This recommendation may be hastened by elevating environmental temperatures or slowed at lower environmental temperatures. Also, accounting for the thermal transfer properties of bags and pellets would have to be accounted for to ensure interior of bags or pallets to ensure adequate inactivation. Again, this is an area that may need more investigation.

1.3 Irradiation

1.3.1 Sunlight

The TGEV is highly photosensitive where 10^5 infectious doses in feces were inactivated with sunlight for 6 hours (Panseart, 1999).

1.3.2 UVC irradiation

The UVC irradiation (400 – 315 nm wavelength) technique has been applied in several industries for its germicide effect such as treating water or purification of plasma products

(Caillaet-Fauquet et al., 2004). A UVC dose of 500 J per m^2 resulted in a greater than 4 log reduction factor for TGEV in 10 to 30% platelet concentrates (Terpstra et al., 2008).

Unfortunately, exposure to sunlight or UVC has low penetrating power thus their sterilizing ability is only on the surface. Components of feces and feed ingredients surrounding the virus therefore obstruct disinfection and leads to a low probability of effectiveness.

1.3.3 Ionizing radiation

Three different kinds of rays: gamma rays (generated from cobalt-60 or cesium-137), electron beams, and x-rays are permitted for treatment of food by FDA with dose limits from 1 to 44 kGy depending on type of food. The limit doses are claimed to be able to control several organisms such as *Salmonella*, *Vibrio,* parasite, or insect pests. However, the smaller nucleic acid of viruses takes more irradiation to inactivate. Nims and associates (2011) developed a relationship between viral particle size (nm for midpoint of range) or viral genomic size (nucleotides) and efficacy of inactivation viruses in frozen bovine serum by gamma radiation shown in the following figures.

Relationship between Viral Particle Size or Genomic Size and Efficacy of Inactivation Viruses by Gamma Irradiation (from Nims et al, 2011)
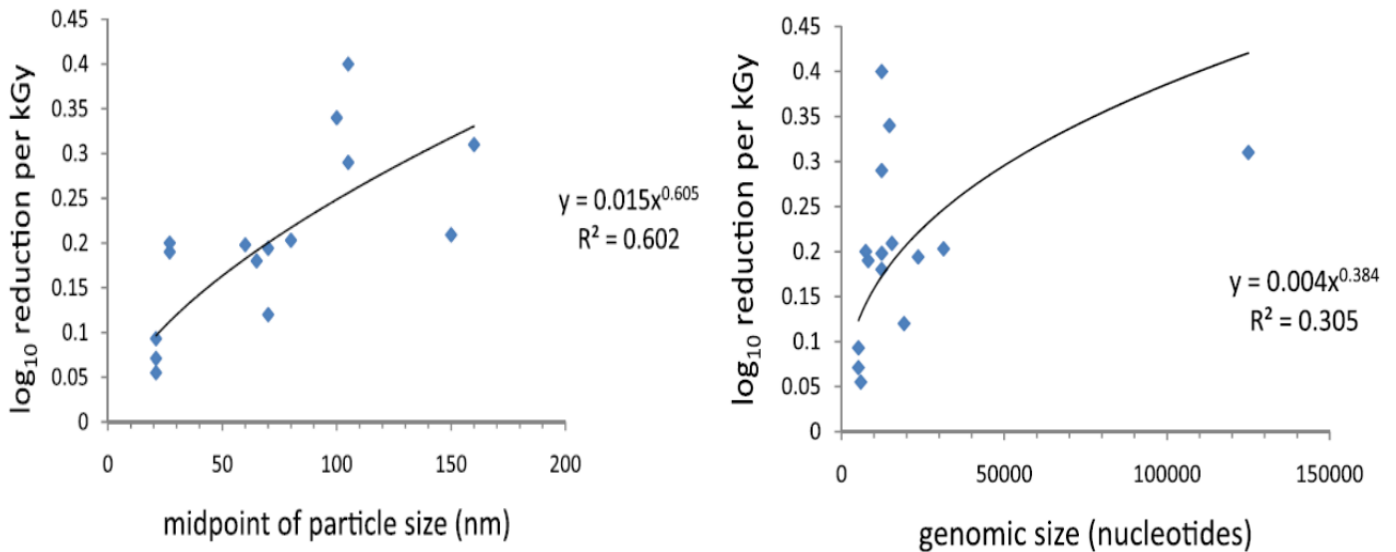
Using the data of Nims et al. (2011), 1 kGy of irradiation will theoretically result in a 0.20 log reduction active PEDV based on particle size (100 nm) or a 0.16 log reduction based on genomic (25,000 nucleotides) size of PEDV. This is consistent with the inactivation dosages for Parainfluenza type 3 (PI3; 0.202 log $_{10}$ reduction per kGy) and bovine viral diarrhea virus (BVDV; 0.198 log $_{10}$ reduction per kGy) which have similar nucleic acid properties as PEDV, medium size, single-stranded, enveloped RNA (Nims et al., 2011).

From this theoretical relationship, 1 ml of feces that contains 10^8 to 10^{10} PEDV (10 to 12 cycle time) would need 40 to 50 kGy to completely inactivate all the viruses. However, diagnostic testing of contaminated feed with PEDV has been resulted in 30 to 33 cycle times which is approximately equivalent to 10^2 to 10^3 PEDV particles (3 cycle time for 10-fold dilution). Thus, the gamma radiation dosage possibly needed to inactivate PEDV in feed is 10 to 15 kGy (using 0.20 log reduction of PEDV per kGy).

2 Feed additives

2.1 Acids & Alkalis

Acids destroy nucleic acid bonds and precipitate protein as well as changing the pH of the environment to destroy microorganisms (Dvorak 2008). Pospischil et al. (2002) reported that PEDV is stable in pH 5 to 9 at 39°F (4°C) or pH 6.5 to 7.5 at 98.6 °F (37°C). Also, TGEV was reported to be stable at pH 3 (Saif et al, 2012; Pansaert 1999). From this information, it seems that PEDV can survive in a broad range of pH in a cold environment and probably in a quite acidic condition similar to TGEV. Therefore, we believe a pH reducer is unlikely to be efficacious for inactivating PED virus in feed.

Alkali agents saponify lipids within envelops of microorganisms resulting in a microbicidal property (Dvorak, 2008). Since PEDV is an enveloped virus, this is the mechanism responsible for effectiveness of household bleach for disinfecting the virus. Sodium hydroxide including other alkalis such as sodium carbonate, calcium oxide, potassium hydroxide, magnesium hydroxide, ammonium hydroxide are generally recognized as safe (GRAS) by FDA and thus can be added to animal feeds as nutritional dietary supplements. Sodium hydroxide has been proven to be able to inactivate TGEV (Saif et al., 2012). A 6.5-log reduction in TGEV titer was reported after a 5-minute incubated with 5% sodium hydroxide (Brown, 1981). Careful

handling procedures must be implemented, as sodium hydroxide is a strong alkali and thus can cause corrosion of metal and wood as well as threat to personnel safety. We were unable to locate information for the other alkalis on their ability to inactivate swine coronaviruses but assume they have a potential for effectiveness. Unfortunately, strong alkali is readily inactived in an organic matrix which may severely limit their effectiveness in feed.

2.2 Formaldehyde

The FDA lists 37% formaldehyde (aqueous solution) as a food additive permitted in feed or drinking water of animals (CFR 573.460) with a dosage of 5.4 lbs (2.5 kg) per ton of animal feed or feed ingredient. This is equivalent to 0.1% pure formaldehyde (0.27% of 37% formaldehyde) in the feed. There is little information on efficacy of formaldehyde to control viruses in feed. In the review of PEDV by Pospischil et al. (2002), PEDV can be inactivated by exposure to 1% formalin. This dosage is much greater than that is recommended (0.03% formalin) to inactivate TGEV in the review by Saif et al. (2012). Guidelines on disinfectants for healthcare facilities by CDC (2008) indicates 2% formalin was inactivates most of viruses. Apparently, formaldehyde could potentially be an alternative to reduce contamination of PEDV through using as a feed additive or as feed mill disinfectant; however, the concentration is not conclusive. Proper application and thoroughly mixing QA/QC practices need to be employed to ensure adequate treatment of the complete feed or ingredients. Because formaldehyde is recognized as an antimicrobial agent to maintain *Salmonella* negative feed by FDA, the label must display the statement "Treated with formaldehyde to maintain feed *Salmonella* negative. Use within 21 days". A major draw back of formaldehyde is the safety of mill personnel during application. Currently, there are human safety regulations (OSHA, SARA) to handle formaldehyde that need to be rigorously followed. A commercial formaldehyde based product is available for addition to feed or feed ingredients (Termin-8, www.anitox.com).

3 Control of carrier

The study by Kamua et al. (2010) reported that PEDV cannot replicate in mice. Cats, dogs, and foxes can shed TGEV in feces. Also, starlings and houseflies can excrete TGEV after being fed with the virus (Saif et al., 2012). These carriers thus need to be monitored to reduce the viral contamination but do not appear to be a primary means of corona virus transmission.

<u>Summary</u>

The recent introduction of PEDV into the North American swine industry has resulted in an urgent need to define protocols that will stop its transmission. Even though it has been present in Europe and Asia for over 40 years, there is little information available that describes procedures of controlling the spread of the virus. Because PEDV is a coronavirus similar to TGEV, procedures used to control TGEV could potentially be effective in the control of PEDV transmission. Currently these "best estimates" based on past TGEV experiences are recommended; however, research urgently needs to be conducted to verify their effectiveness against the PEDV.

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