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Effect of Thermal Mitigation on Porcine Epidemic Diarrhea Virus (PEDV)-Contaminated Feed[1]

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Summary

Porcine Epidemic Diarrhea Virus (PEDV) is primarily transmitted by fecal-oral contamination. However, epidemiological evidence has shown that swine feed and ingredients may serve as potential vectors of transmission. Since it is known that PEDV is a heat-sensitive virus, we hypothesized that a conditioner and pellet mill mimicking commercial thermal processing would mitigate PEDV infectivity. To test this hypothesis, two experiments were designed to determine if different pellet mill conditioner retention times or temperatures would impact PEDV infectivity determined by polymerase chain reaction (PCR) analysis and bioassay. For the first study, a 3×3×2 factorial was utilized, with three pelleting temperatures (155, 175, or 195°F), three conditioning times (45, 90, or 180 s), and two levels of virus (low: 1×10^2 $TCID_{50}$/g, or high: 1×10^4 $TCID_{50}$/g). Non-inoculated and PEDV-inoculated unprocessed mash were used as controls. There was no PEDV RNA detected in the PEDV-free mash. The low-dose PEDV-infected mash was 6.8 ± 1.8 cycle threshold (Ct) greater ($P < 0.01$) than the high dose mash. Regardless of time or temperature, feed processing increased ($P < 0.01$) the Ct compared to the PEDV-inoculated unprocessed mash. As expected, fecal shedding of PEDV was not detected in rectal swabs from control pigs for the duration of the study. Fecal swabs from pigs fed the PEDV-inoculated unprocessed mash, regardless of dose, were PEDV-positive from 2 to 7 days post-inoculation, at which time the pigs were sacrificed. However, if either PEDV dose of inoculated feed was pelleted at any of the nine tested conditioning time × temperature combinations, no PEDV RNA was detected in fecal swabs or cecum content. Based on these results, a second experiment was developed to determine the impact of lower processing temperatures on PEDV infectivity. The pellet mill was heated for 1 hour at normal manufacturing conditions

[1] Appreciation is expressed to the National Pork Board for financial support (awards #14-159 and #14-273).

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prior to simulating a plug by turning off the steam supply. This allowed the temperature of the mash feed to decrease below 100°F. The PEDV-inoculated feed was then pelleted at one of five conditioning temperatures (100, 115, 130, 145, or 160°F) for 30 s. This study was repeated three times on three separate days with complete decontamination between each experiment day. Again, non-inoculated and PEDV-inoculated mash were used as controls. The five increasing temperatures led to feed with respective mean Ct values of 32.5, 34.6, 37.0, 36.5, and 36.7. Even though all samples had detectable PEDV RNA in the feed, infectivity was only detected by bioassay in pigs from the 100 and 115°F conditioning treatments. In each of the other processing temperatures, no PEDV RNA was detected in fecal swabs or cecum contents.

Our results suggest that processing feed through a conditioner and pellet mill similar to those used in commercial feed mills will be effective as a point-in-time mitigation step for PEDV as long as conditioning temperatures remain above 130°F. Any time feed is processed at temperatures below that level, such as during start-up or when the pellet mill die becomes plugged and the steam is consequently shut off, there is a risk that the feed can act as a vector for transferring infectious PEDV and lead to cross-contamination of post-pelleting handling equipment.

Key words: feed safety, PEDV, thermal mitigation, swine

Introduction

In a review by Nitikanchana (2014[5]), a theoretical temperature × time relationship was proposed to reduce the infectivity of PEDV in complete feeds based on data extrapolated primarily from PEDV environmental survival studies. Typical swine feed pelleting conditioner retention times and temperatures encompass the theoretical temperature × time relationship proposed. We are unaware of any available direct research confirming this time × temperature relationship using conditioner and pellet mills that are present in modern feed manufacturing facilities.

Although it would be highly uncommon to set target pellet mill conditioning temperatures below 155°F, it is possible that feed may be produced below these operating limits. This commonly would occur during start-up of the pellet mill or during a pellet mill die plug, which is often resolved without manual intervention by turning off steam to the conditioner in an effort to unclog the die. This can lead to significant quantities of pelleted feed that has not reached the target conditioning temperature during production. If PEDV particles were in the feed, the resulting feed conditioned at a lower temperature may still have infectious PEDV particles that may potentially contaminate the pellet cooler; post-pellet feed handling equipment and bins at the feed mill; trucks; and bins, feed lines, and feeders at the farm. The objectives of this study were to 1) determine the impact of conditioning time and temperature of pelleted complete feed at two PEDV dosages on PEDV inactivation as measured by RT-PCR and bioassay, and 2) determine the efficacy of increasing pellet mill conditioning temperature on PEDV-survivability as measured by qRT-PCR (quantitative reverse transcription PCR) and bioassay.

[5] Nitikanchana, S. 2014. Potential Alternatives to Reduce Porcine Epidemic Diarrhea Virus (PEDV) Contamination in Feed Ingredients. http://www.asi.k-state.edu/species/swine/research-and-extension/PEDV%20contamination%20in%20feed%20ingredients_Feb%2026.pdf (Accessed 31 December 2014).

Materials and Methods

The feed used was a corn soybean meal-based swine diet (Table 1) manufactured at the Kansas State University O. H. Kruse Feed Technology Innovation Center in Manhattan, Kansas. A subsample of this feed was obtained prior to inoculation and confirmed negative by real-time PCR (RT-PCR) for the presence of PEDV RNA at the Kansas State University Research Park Molecular Diagnostics Development Laboratory in Manhattan, Kansas.

Experiment 1

The low dose (20 Ct) and high dose (13 Ct) concentrations in the feed were used based on the results obtained from previous research.[6] Within each dose, the batches of inoculated feed were processed at one of three condition times (45, 90, 180 sec) with one of three processing temperatures (155, 175, and 195°F). Thus, treatments were arranged in a 2 (PEDV dose) × 3 (conditioning time) × 3 (processing temperature) arrangement. Additionally, three batches of unprocessed (meal based) feed were used as control treatments. These batches consisted of feed inoculated with virus-free tissue culture medium, feed inoculated with the low dose PEDV, and feed inoculated with the high dose PEDV concentration.

Conditioning times were chosen to represent a typical conditioning time, an extended time using a typical conditioner, and an extended time that would require a modified conditioner. The temperature range reflects a typical nursery diet processing temperature on the low end and the maximum achievable temperature under typical commercial production of swine feed on the high end.

For each concentration, aliquots (17 oz) of the PEDV dilutions were uniformly blended into 10-lb batches of feed. Feed from each of the conditioning time × temperature combinations (9 batches within each PEDV dose) was processed using a pilot-scale single pass conditioner and pellet mill (Model CL5, CPM, Waterloo, IA). Prior to pelleting the first batch, non-inoculated feed was processed until the exit temperature of the feed was stable at the target temperature. In between each inoculated batch, a minimum of 11 lb of virus-free feed was processed. The objective of this was two-fold. The first objective was to provide a flush of the equipment and prevent virus carryover. The second was to stabilize the pelleting temperature so the contaminated feed was processed under uniform temperature conditions. The low-dose batches were pelleted prior to the high-dose batches. Within each dose, the highest temperature and longest retention time was pelleted first, and then the temperature was reduced to achieve the medium- and low-temperature batches before moving to the middle retention time. The same progression was used between the medium- and low-retention time, such that the final batch within each dose was the low temperature and shortest retention time treatment.

Three 0.22-lb. samples of each feed batch were added to 13.5 oz of cold phosphate buffered saline (PBS, pH 7.4) in 17-oz. bottles, thoroughly mixed and stored at 39.2°F for

[6] Woodworth, J. C., C. R. Stark, R. A. Hesse, R. G. Main, J. Zhang, M. D. Tokach, P. C. Gauger, and S. S. Dritz. 2014. Determining the impact of conditioning time and temperature in pelleted diets on porcine epidemic diarrhea virus (PEDV) survivability in complete swine diets- NPB#14-159. http://research.pork.org/FileLibrary/ResearchDocuments/14-159-WOODWORTH-KSt.pdf (Accessed 14 March 2015.

approximately 12 h. The feed suspension supernatants were then collected and evaluated using a PEDV N-gene based RT-PCR and aliquots were harvested and frozen at -112°F until use in the pig bioassay.

Experiment 2

A subsample of the feed was obtained prior to inoculation for each replication and confirmed negative by qRT-PCR for the presence of PEDV RNA at the Kansas State University Research Park Molecular Diagnostics Development Laboratory in Manhattan, Kansas. The negative control treatment was formed by mixing a 110-lb batch of the swine diet in a 0.14-yd^3 electric paddle mixer (H. C. Davis Sons Manufacturing, model# SS-L1; Bonner Springs, KS). A mixing time of 5 min was determined to achieve a uniform mix with a coefficient of variation of less than 10%.

A 17-oz aliquot of stock PEDV was mixed into a 10-lb batch of swine feed using procedures established in a prior experiment.[6] Briefly, feed and virus were mixed using a manual, bench-top laboratory scale stainless steel paddle mixer previously validated for mixer efficiency. The stock virus was slowly added during mixing, and a wet mix time of 2.5 min was used to confirm a consistent PEDV-contaminated mix, which served as the PEDV feed inoculum.

The PEDV feed inoculum (10 lb of feed + 17 oz. of stock virus) was added to 99.2 lb of PEDV-free swine diet to form the positive control in the same 0.14-yd^3 electric paddle mixer, and the entire batch of positive control feed was then mixed for 5 min, discharged for 10 min into biohazard containers, and finally held at 28.4°F until the start of the thermal processing portion of the study.

PEDV-free feed was pelleted using the same pilot-scale single-pass conditioner and pellet mill (Model CL5, CPM, Waterloo, IA). The pellet mill was heated by pelleting PEDV-free feed with a conditioning temperature of 160°F for 60 min. The steam valve was then turned off until the conditioning temperature dropped below 98.6°F to mimic procedures commonly used to resolve a plug in the conditioner or pellet die. Next, PEDV-inoculated feed was removed from the freezer and added to the pellet mill hopper. Once PEDV-inoculated feed started passing through the pellet mill, steam was slowly added, and five pelleted samples were collected at targeted hot mash conditioner temperatures of 100, 115, 130, 145, and 160°F (± 2.2°F) with feed pelleted using a 30-sec conditioning time. These conditioning temperatures were selected based on a previously determined prediction equation for the specific pellet mill to result in hot pellet temperatures of 104, 125.6, 145.4, 163.4, and 176°F, respectively.

Bioassay

The Iowa State University Institutional Animal Care and Use Committee reviewed and approved the pig bioassay protocol. Eighty-one crossbred, 10-d-old pigs (33 pigs for Exp. 1 and for 48 pigs for Exp. 2) of mixed sex were sourced from a single commercial, crossbred farrow-to-wean herd with no prior exposure to PEDV. Upon arrival, piglets were ear tagged, weighed, and administered a dose of cefitiofur (Excede, Zoetis, Florham Park, NJ). Also upon arrival, fecal swabs were obtained and confirmed negative for PEDV, porcine delta coronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV) using a RT-PCR assay. To further confirm PEDV-negative status, serum

was collected and confirmed negative for PEDV antibody by an indirect fluorescent antibody (IFA) assay and transmissible gastroenteritis virus (TGEV) antibody by enzyme-linked immunosorbent assay (ELISA) conducted at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Pigs were allowed 2 d of adjustment to the new pens before the bioassay began.

In Exp. 1, a total of 22 rooms (66 pigs) were randomly assigned to treatments corresponding to the 2 x 3 x 3 factorial design, with each room representing each treatment. In Exp. 2, a total of 16 rooms (48 pigs) were assigned to treatment groups with 1 negative control room and 15 challenge rooms. Each pig from the negative control room was given a 10-mL aliquot of inoculum created from the PEDV-free feed collected from the electric mixer during each of replicate 1, 2, and 3. Different from the negative control room, each pig in each challenge room was given an aliquot of inoculum from the same replicate, and one room was representative of a treatment in a single replicate.

In both experiments, rectal swabs were collected on d -2, 0, 2, 4, 6, and 7 post inoculation (dpi) from all pigs and tested for PEDV RNA by quantitative qRT-PCR. Pigs were humanely euthanized at 7 dpi, and fresh small intestine, cecum, and colon samples were collected at necropsy, along with an aliquot of cecal content. One section of formalin-fixed proximal, middle, distal jejunum, and ileum was collected per pig for histopathology (Chen et al., 2014[7]). Infectivity of cecal content was evaluated for PEDV by qRT-PCR.

Statistical Analysis

Statistical analysis was carried out using SAS (SAS Institute, Inc., Cary, NC). In Exp 1, ANOVA was performed to evaluate PEDV RNA feed Ct values, villus height, crypt depth, villus height to crypt depth ratio, and immunohistochemistry. In Exp. 2, data of the effects of conditioner temperature on villus height, crypt depth, and villus height to crypt depth ratio were analyzed as a completely randomized design using PROC MIXED in SAS (SAS Institute, Inc., Cary, NC) with pig as the experimental unit by a pairwise comparison. Results for treatment criteria were considered significant at $P \leq 0.05$ and marginally significant from $P > 0.05$ to $P \leq 0.10$.

Results and Discussion

Experiment 1

No PEDV RNA was detected in the unprocessed PEDV-free feed. When the low-dose PEDV medium (Ct 20) was mixed with the feed, the resulting feed Ct value was 31, and when the high-PEDV dose medium (Ct of 13) was mixed with feed, the resulting Ct value was 24 (Table 2).

As expected, fecal shedding of PEDV was not detected in rectal swabs from negative control pigs for the duration of the study (Table 2). Fecal swabs from pigs fed the low- and high-PEDV dose positive control treatment (inoculated, but nonprocessed feed) were PEDV-positive from 2 dpi through the end of the study at 7 dpi. Cecum contents

[7] Chen, Q., G. Li, J. Stasko, J. T. Thomas, W. R. Stensland, A. E. Pillatzki, P. C. Gauger, K. J. Schwartz, D. Madson, K. J. Yoon, G. W. Stevenson, E. R. Burrough, K. M. Harmon, R. G. Main, and J. Zhang. 2014. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. J. Clin. Microbiol. 52: 234-243.

at 7 dpi and IHC determined 7 dpi were also positive for the positive control pigs (Table 2). However, if either the low- or high-dose PEDV feed was processed at any of the 9 possible conditioning time × temperature combinations, no PEDV RNA was detected in fecal swabs or cecum contents at 7 dpi.

The villus height for pigs challenged with the non-inoculated feed was higher ($P < 0.01$) compared to the height in pigs challenged with the low- or high-dose PEDV unprocessed feed (Table 3). Porcine Epidemic Diarrhea Virus IHC immunoreactivity was not visible in the cytoplasm of villus enterocytes of low- or high-dose challenged pigs from any of the time and temperature pellet treatment combination for the duration of the study.

Experiment 2

When PEDV-inoculated feed was processed at five different conditioning temperatures (100, 115, 130, 145, and 160°F), the respective mean cycle threshold (Ct) values as detected by qPCR were 32.5, 34.6, 37.0, 36.5, and 36.7, respectively (Table 4). All 9 of the feed samples conditioned at 100, 115, or 130°F had detectable PEDV RNA; whereas 8 of the 9 processed at 143.6 or 159.8°F had detectable PEDV RNA. It was observed that Ct value increased as conditioning temperature increased to 130°F, with little change thereafter.

As in Exp. 1, fecal shedding of PEDV was not detected in rectal swabs or cecum contents from pigs fed the PEDV-negative control for the duration of the study (Table 5). Of the 9 total pigs gavaged with aliquots from the PEDV-positive diet conditioned at 100°F, a fecal swab from 1 pig (Room 7, Replicate 2) yielded detectable PEDV RNA at 2 dpi, and the fecal swabs and cecum contents of all 3 pigs in Room 7 had detectable viral particles by 4 through 7 dpi. In addition, 3 pigs gavaged with aliquots from the treatment conditioned at 115°F had detectable fecal PEDV RNA at 2 to 7 dpi, and all pigs were in the same room (Room 8, Replicate 2). No pig challenged with feed conditioned at or above 130°F had detectable PEDV RNA in fecal swabs or cecum content for the duration of the study.

The pigs challenged with the feed conditioned at 100°F had shorter ($P < 0.05$) villus heights than pigs challenged with any other treatment (Table 6). Furthermore, pigs challenged with feed conditioned at 100, 130, or 145°F had deeper ($P < 0.05$) crypt depths than pigs challenged with the negative control or feed conditioned at 160°F. This led to pigs challenged with feed conditioned at 100°F to have a lower ($P < 0.05$) villus height:crypt depth ratio than pigs challenged with the negative control or feed conditioned at 130, 145, or 160 °F. Porcine Epidemic Diarrhea Virus IHC immunoreactivity was visible in the cytoplasm of villus enterocytes of pigs challenged with the two lowest processed temperatures, 100 and 115°F, when harvested at 7 dpi.

In summary, infected feed processed at common manufacturing temperatures and conditioner retention times effectively inactivated the PED virus at a low and high concentration and did not cause detectable clinical or microscopic disease in 10-d-old pigs. This suggests that thermal processing is one step that can be used to reduce the risk of PEDV transmission. If target processing temperatures are not reached, such as during resolution of a plugged pellet mill, a risk occurs of transmitting PEDV through con-

taminated pelleted feed. Our data suggest that feed should be conditioned at temperatures above 130°F to minimize the risk of PEDV transfer. Thus, care should be taken if suspected PEDV-contaminated feed is conditioned below the target temperature to prevent downstream cross-contamination.

Table 1. Diet composition used in Exp. 1 and 2

Item	Negative control
Ingredient, %	
Corn	79.30
Soybean meal, 46.5 CP	15.70
Choice white grease	1.00
Monocalcium phosphate	1.40
Limestone, ground	1.15
Salt	0.50
L-Threonine	0.03
Trace mineral premix[1]	0.15
Sow add pack[2]	0.50
Vitamin premix[3]	0.25
Phytase[4]	0.02
Total	100.00
Formulated analysis, %	
Dry matter	91.4
Crude protein	17.1
Crude fiber	3.7
Ether extract	3.5
Ca	0.78
P	0.52

[1] Each kilogram contains 26.4 g Mn, 110 g Fe, 110 g Zn, 11g Cu, 198 mg I, and 198 mg Se.
[2] Each kilogram contains 220,000 mg choline, 88 mg biotin, 660 mg folic acid, 1,980 mg pyridoxine.
[3] Each kilogram contains 4,400,000 IU vitamin A, 660,000 IU vitamin D_3, 17,600 IU vitamin E, 1,760 mg menadione, 3,300 mg riboflavin, 11,000 mg pantothenic acid, 19,800 mg niacin, 15.4 mg vitamin B_{12}.
[4] High Phos 2700 GT, DSM Nutritional Products, Parsippany, NJ.

Table 2. Effects of Porcine Epidemic Diarrhea Virus (PEDV) dose, pelleting temperature, and conditioning retention time on PEDV detection from feed, pig fecal swabs, and cecum contents, Exp. 1[1]

		PEDV N-gene Real Time-PCR, cycle threshold (Ct)							
				Fecal swabs					Cecum contents
PEDV dose, temp.[3], and time[4]		Tissue culture	Feed	0 dpi[2]	2 dpi	4 dpi	6 dpi	7 dpi	7 dpi
Unprocessed virus-free feed[5]		–	–	–	–	–	–	–	–
Low dose inoculated feed[6]		20.0	31.0	–	22.4	18.2	18.8	24.1	26.7
155°F	45s		42.6	–	–	–	–	–	–
	90s		39.5	–	–	–	–	–	–
	180s		45.0	–	–	–	–	–	–
175°F	45s		36.7	–	–	–	–	–	–
	90s		39.7	–	–	–	–	–	–
	180s		42.3	–	–	–	–	–	–
195°F	45s		39.7	–	–	–	–	–	–
	90s		37.4	–	–	–	–	–	–
	180s		35.9	–	–	–	–	–	–
High dose inoculated feed[7]		13.0	24.0	–	23.0	15.3	20.4	24.3	24.0
155°F	45s		30.2	–	–	–	–	–	–
	90s		29.7	–	–	–	–	–	–
	180s		30.2	–	–	–	–	–	–
175°F	45s		30.1						
	90s		29.5	–	–	–	–	–	–
	180s		30.2	–	–	–	–	–	–
195°F	45s		30.1						
	90s		30.6	–	–	–	–	–	–
	180s		30.0	–	–	–	–	–	–

[1] An initial tissue culture containing a low dose and high dose of PEDV was used to inoculate batches of feed. Three feed samples per batch were collected and diluted in PBS. The supernatant from each sample was then collected for pig bioassay. The supernatant was administered one time via oral gavage on d 0 to each of three pigs per treatment (10 ml per pig). Thus, each value represents the mean of three pigs per treatment. Pigs were initially 10 d old and 7.9 lb.

[2] Day post inoculation.

[3] Temperature of feed exiting the conditioner.

[4] Retention time. The amount of time required for feed to pass through the conditioner.

[5] A cycle threshold (Ct) of >45 was considered negative for presence of PEDV RNA.

[6] For low-dose feed, PEDV (1×10^3 $TCID_{50}$/ml) was diluted into feed to provide a dose of 1×10^2 $TCID_{50}$/g of feed.

[7] For high-dose feed, PEDV (1×10^5 $TCID_{50}$/ml) was diluted into feed to provide a dose of 1×10^4 $TCID_{50}$/g of feed.

Table 3. Effects of Porcine Epidemic Diarrhea Virus (PEDV) dose, pelleting temperature, and conditioning retention time on morphologic and immunohistochemistry evaluation of small intestine from pigs, Exp. 1[1]

		Morphology[2]			
Item		Villus height, mm	Crypt death, mm	Villus height to crypt depth ratio	Immunohistochemistry (IHC)[3]
Unprocessed virus-free feed		495.7	101.7	4.9	0
Low dose inoculated feed[4]		414.3	91.0	4.6	0.3
155°F	45s	481.6	101.3	4.8	0
	90s	489.3	108.1	4.6	0
	180s	504.4	115.6	4.4	0
175°F	45s	508.6	108.9	4.7	0
	90s	476.4	103.6	4.6	0
	180s	441.8	103.6	4.3	0
195°F	45s	443.2	97.8	4.6	0
	90s	495.7	101.7	4.9	0
	180s	441.8	103.6	4.3	0
High dose inoculated feed[7]		309.3	112.6	3.1	1.7
155°F	45s	423.1	105.3	4.0	0
	90s	429.4	118.3	3.7	0
	180s	389.2	100.0	4.0	0
175°F	45s	432.8	117.3	3.7	0
	90s	390.6	102.5	3.7	0
	180s	448.7	104.5	4.3	0
195°F	45s	383.1	119.5	3.2	0
	90s	446.4	102.8	4.4	0
	180s	408.9	105.7	3.9	0
	SEM	29	10.1	0.4	0.2

[1] An initial tissue culture containing a low dose or high dose of PEDV was used to inoculate batches of feed. Three feed samples per batch were collected and diluted in PBS. The supernatant from each sample was then collected for pig bioassay. The supernatant was administered one time via oral gavage on d 0 to each of three pigs per treatment (10 ml per pig). Thus, each value represents the mean of three pigs per treatment necropsied at 7 d post infection. Pigs were initially 10 d old and 7.9 lb.

[2] Intestinal cross-sections were fixed in formalin and stained with hematoxylin and eosin for evaluation.

[3] Three sections of ileum were evaluated and averaged into one categorical value per pig. Categorical values were assigned for each pig (0=no signal, 1=mild, 2=moderate, 3=abundant, 4=diffuse) and reported as the mean from three pigs per treatment, thus the mean of 9 values.

[4] For low-dose feed, PEDV (1×10^3 $TCID_{50}$/ml) was diluted into feed to provide a dose of 1×10^2 $TCID_{50}$/g of feed.

[5] Temperature of feed exiting the conditioner.

[6] Retention time. The time feed was inside the conditioner.

[7] For high-dose feed, PEDV (1×10^5 $TCID_{50}$/ml) was diluted into feed to provide a dose of 1×10^4 $TCID_{50}$/g of feed.

Table 4. Effect of increasing pelleting temperature on Porcine Epidemic Diarrhea Virus (PEDV)-infected feed as analyzed by qRT-PCR (quantitative reverse transcriptase PCR), Exp. 2[1]

	Mash conditioner temperature				
Positive treatment[2]	100°F	115°F	130°F	145°F	1160°F
% positive[3]	100.0 (9/9)	100.0 (9/9)	100.0 (9/9)	88.9 (8/9)	88.9 (8/9)
Cycle threshold (Ct)[4]	32.5	34.6	37.0	36.5	36.7

[1] 17 oz of tissue culture containing 4.5×10^6 $TCID_{50}$/ml of PEDV was inoculated into a 10-lb batch of feed, then added to 110 lb of PEDV-negative feed to form the positive treatment. One feed sample per temperature per replicate × 3 replicates was collected, divided into 3 aliquots, and diluted in PBS to form supernatants. Thus each feed supernatant value per treatment represents the mean of three replicates × 3 repetitions.
[2]Pellet mill die was warmed-up for approximately 1 hour before the steam was turned off. Steam was slowly added and the five processed samples were collected.
[3] Means represent the percent of samples that had detectable RNA by PEDV qPCR analysis.
[4] Mean cycle threshold (Ct) value of samples with detectable PEDV RNA below 45.

Table 5. Influence of processed Porcine Epidemic Diarrhea Virus (PEDV)-inoculated feed on qRT-PCR (quantitative reverse transcriptase PCR) cycle threshold (Ct) of feed, fecal swabs, and cecum contents of pigs Exp. 2[1]

		Fecal swabs, Ct					
Item	KSU feed inoculum, Ct	0 dpi[2]	2 dpi	4 dpi	6 dpi	7 dpi	7 dpi Cecum content, Ct
Processed feed, %[3]							
Negative	-[4]	-	-	-	-	-	-
100°F	100.0 (9/9)	-	11.1 (1/9)	33.3 (3/9)	33.3 (3/9)	33.3 (3/9)	33.3 (3/9)
115°F	100.0 (9/9)	-	33.3 (3/9)	33.3 (3/9)	33.3 (3/9)	33.3 (3/9)	33.3 (3/9)
130°F	100.0 (9/9)	-	-	-	-	-	-
145°F	88.9 (8/9)	-	-	-	-	-	-
160°F	88.9 (8/9)	-	-	-	-	-	-
Processed feed, Ct[5]							
Negative	-	-	-	-	-	-	-
100°F	32.5	-	15.8	27.5	16.6	17.8	16.9
115°F	34.6	-	24.5	15.2	15.4	17.9	18.8
130°F	37.0	-	-	-	-	-	-
145°F	36.5	-	-	-	-	-	-
160°F	36.7	-	-	-	-	-	-

[1] 17 oz. of tissue culture containing 4.5×10^6 $TCID_{50}$/ml of PEDV was inoculated into a 10-lb batch of feed, then added to 110 lb of PEDV negative feed to form the positive treatment. One feed sample per temperature per replicate × 3 replicates was collected, divided into three aliquots, and diluted in PBS to form supernatants. Thus each feed supernatant value per treatment represents the mean of three replicates × 3 repetitions.
[2] Day post inoculation.
[3] Means represent the percent of samples that had detectable RNA by PEDV qPCR analysis (< 45 Ct).
[4] No detectable PEDV RNA (Ct >45).
[5] Mean cycle threshold (Ct) value of samples with detectable PEDV RNA below 45.

Table 6. Morphologic and immunohistochemistry evaluation of small intestine from pigs that were challenged with Porcine Epidemic Diarrhea Virus (PEDV)-inoculated feed processed at increasing temperatures, Exp. 2[1]

	Morphology[2]			
Item	Villus height, mm	Crypt depth, mm	Villus height to crypt depth ratio	Immunohistochemistry (IHC)[3]
Processed feed				
Negative	395.3±27.1[a]	245.1±18.3[a]	2.1±0.2[b]	0
100°F	299.8±15.6[b]	188.2±10.6[b]	1.6±0.1[a]	0.7
115°F	374.4±15.6[a]	217.0±10.[6ab]	1.7±0.1[ab]	0.6
130°F	380.3±15.6[a]	190.9±10.6[b]	2.0±0.1[b]	0
145°F	387.3±15.6[a]	199.5±10.6[b]	1.9±0.1[b]	0
160°F	385.9±15.6[a]	218.8±10.6[a]	1.8±0.1[b]	0

[1] 17 oz of tissue culture containing 4.5×10^6 $TCID_{50}$/ml of PEDV was inoculated into a 10-lb batch of feed, then added to 110 lb of PEDV-negative feed to form the positive treatment. One feed sample per temperature per replicate × 3 replicates was collected, divided into three aliquots, and diluted in PBS to form supernatants. Thus each feed supernatant value per treatment represents the mean of 3 replicates × 3 repetitions.

[2] Intestinal cross-sections were fixed in formalin and stained with hematoxylin and eosin (H&E) for evaluation.

[3] Three sections of ileum were evaluated and averaged into one categorical value per pig. Categorical values were assigned for each pig (0=no signal, 1=mild, 2=moderate, 3=abundant, 4=diffuse) and reported as the mean from 3 pigs per negative treatment and mean from 9 pigs per remaining treatments.

[a,b] Means within column lacking a common superscript are different ($P < 0.05$).