RESEARCH ABSTRACT
SWINE HEALTH

Title: Utilizing feed sequencing as a biosecurity intervention for preventing PEDV cross-contamination in the feed manufacturing process. – **NPB #14-273**

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SCIENTIFIC ABSTRACT

This study aimed to utilize the only known pilot feed mill facility approved for pathogenic feed agent use in the United States to 1) determine if feed sequencing could minimize the risk of PEDV cross-contamination; 2) evaluate the effect of conditioning PEDV-contaminated feed below traditional pelleting temperatures on PEDV as measured by PCR and bioassay; and 3) evaluate the effect of manufacturing PEDV-contaminated feed on subsequent feed mill environmental surface contamination. Two simultaneous experiments were conducted to achieve these objectives. In Exp. 1, a PEDV-free corn, soybean meal-based swine diet was manufactured in a 0.11 m^3 electric paddle mixer to represent the negative control. Feed was discharged from the mixer, conveyed through a pilot-scale bucket elevator, and discharged into segregated biohazard containers. Negative control samples were collected at the mixer prior to discharge and at the bucket elevator during discharge. Next, a 500 ml aliquot of PEDV isolate (USA/IN/2013/19338 P7) with a PCR cycle threshold value (Ct) of 11 was uniformly mixed into 4.5 kg of the complete swine diet using a manual stainless steel benchtop paddle mixer. The PEDV-inoculated feed was then added to 45 kg of complete swine diet and mixed using the 0.11 m^3 electric paddle mixer to create the PEDV-contaminated feed treatment that served as the positive control. The positive control was then sampled and discharged similar to that described for the negative control. Four subsequent 50-kg batches (Sequence 1 to 4) of the PEDV-free swine diet were sequenced through the electric mixer, bucket elevator, and discharged with samples collected from each Sequence as previously described. All samples were analyzed for presence of PEDV genetic material by quantitative PCR (qPCR). The samples from the positive control, Sequence 1, and Sequence 2 were then mixed with PBS, stored overnight, and supernatant was extracted for use in bioassay. In Exp. 2, the positive control treatment was pelleted using a pilot-scale pellet mill previously heated to a 71°C conditioning temperature for one hour. After the positive control feed was added to the hopper, steam was turned off until the conditioner temperature dropped below 37°C to simulate temperature change during the resolution of a pellet die plug. Steam was then added, and five pelleted samples were collected at targeted conditioner temperatures of 37, 46, 54, 62 and 71°C (± 1.2°C) using a 30 to 35 second conditioning time. After production was complete, the pellet mill was swabbed for presence of PEDV RNA detected by qPCR. The pelleted feed was then mixed with PBS overnight before extraction of the supernatant that was subsequently used for bioassay. Both experiments were replicated three times with decontamination of the feed mill and all equipment between replications and at the start and end of the study. As in our previous studies, there was a loss of approximately 10 Ct values when PEDV-cultured media was added to unprocessed mash feed, and an additional 3 Ct loss when feed was diluted 1:1,000. In Exp. 1, there was no PEDV detected from feed samples collected from the mixer during or after Sequence 2 or from the bucket elevator discharge during or after Sequence 3, but all equipment and environmental

surfaces remained positive with detected PEDV genetic material obtained by swabbing, even after Sequence 4. Infectivity resulted from at least one bioassay room per treatment when pigs were challenged with inoculum from the positive control and both Sequence 1 and 2, despite the fact that Sequence 2 feed did not have PEDV RNA detected by qPCR. Additionally, PEDV infection resulted when pigs were fed feed pelleted at the two lowest conditioning temperatures, 37 and 46°C. Environmental swabs indicated widespread surface contamination of the equipment and work area after a PEDV contaminated batch of feed is processed. In summary, sequencing batches of feed should be considered a PEDV risk mitigation strategy, and not a risk elimination strategy. Introduction of PEDV-infected feed into a feed mill will likely result in ubiquitous contamination of equipment and surfaces, even after several batches of PEDV-free feed are produced. If targeted pellet mill conditioning temperatures are not reached, there is risk of transmitting PEDV through contaminated feed. Most concerning, feed without detectible PEDV RNA was demonstrated to be infective. More research is needed to determine if contaminated surfaces are infective and how to best decontaminate a PEDV-contaminated feed mill.