

SWINE HEALTH

Title: Evaluating the prevalence and distribution of African swine fever virus during feed manufacture, as well as feed mill decontamination measures - **_NPB #20-018**

Investigator: Cassandra Jones

Institution: Kansas State University

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Scientific Abstract: It is critical to have methods that can detect and mitigate the risk of African Swine Fever Virus (ASFV) in potentially contaminated feed or ingredients bound for the United States. The purpose of this experiment was to evaluate feed batch sequencing as a mitigation technique for ASFV and if sampling methods could identify ASFV in feed samples following experimental inoculation. Batches of feed were manufactured in BSL-3Ag room at Biosafety Research Institute in Manhattan, Kansas. First, the pilot feed manufacturing system was used to mix, convey, and discharge an ASFV-free diet. Next, a diet was manufactured using the same equipment, but contained feed inoculated with ASFV for a final concentration of 5.6×10^4 TCID₅₀/g. Finally, four subsequent ASFV-free batches of feed were manufactured. After discharging each batch into a collection container, 10 samples were collected in a double 'X' pattern. Environmental swabs from 18 locations were collected after each batch of feed was discharged. The locations of the swabs were categorized into four zones: 1) feed contact surface, 2) non-feed contact surface < 1 meter away from feed, 3) non-feed contact surface > 1 meter from feed, and 4) transient surfaces. Samples were analyzed for ASFV p72 encoding gene PCR assay with response criteria of cycle threshold (Ct) and Log₁₀ genomic copy number (CN)/g of feed. Batch of feed impacted Ct value ($P < 0.0001$) and Log₁₀ genomic CN/g ($P < 0.0001$) of feed samples. Samples after manufacturing the positive control diet contained greatest amounts of detected p72 genetic material across all response criteria ($P < 0.05$). Quantity of detected p72 genetic material decreased sequentially as additional batches of feed were manufactured but still detectable after sequence 4. The sampling method utilized was able to identify ASFV p72 genetic material in samples of feed. For environmental samples, there was no evidence of a zone × batch interaction for log₁₀ genomic CN/mL ($P = 0.625$) or cycle threshold (Ct) value ($P = 0.608$). Sampling zone impacted the log₁₀ p72 genomic CN/mL ($P < 0.0001$) and Ct values ($P < 0.0001$), with a greater amount of viral genome detected on transient surfaces compared to other surfaces ($P < 0.05$). In summary, sequencing batches of feed helps to decrease the concentration of ASFV contamination in feed, but does eliminate it. Bulk ingredients can be accurately evaluated for ASFV by collecting 10 subsamples using the methods described herein to create a common composite sample for analysis. Future research is needed to evaluate using two mitigation techniques in combination to reduce ASFV contamination in feed. Once ASFV enters the feed mill environment it becomes widespread and movement of people can significantly contribute to the spread of ASFV in a feed mill environment.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

For more information contact:

National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org
