Sal CURB® and CaptiSURE™ as Part of a Feed Biosecurity Program\textsuperscript{1,2}

Abstract
Feed has been identified as a risk factor for viral infection in pigs.\textsuperscript{3,4} Products such as Sal CURB® Liquid Antimicrobial have been shown to mitigate this risk.\textsuperscript{4,5} A rigorous, field-based model was used to assess the potential efficacy of mitigants after successful bench-top level and bioassay-based testing. A total of 300 nursery pigs were randomly assigned to 3 treatments and separated into 3 BSL-2 rooms (6 pens/treatment). Treatments consisted of 1) positive control with no feed mitigation, 2) positive control + Sal CURB at 6.5 lb./ton, and 3) positive control + CaptiSURE™ at 20 lb./ton. To infect pigs, a novel model was used placing an ice block into feed, containing 3 viruses (Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Porcine Epidemic Diarrhea Virus (PEDV), and Senecavirus A (SVA)). Pigs in the positive control treatment became infected with all three viruses, whereas pigs in the Sal CURB and CaptiSURE treatments remained free of infection throughout the trial. This study demonstrates the ability of formaldehyde and a medium chain fatty acid blend to prevent a viral infection through contaminated feed.

Materials & Methods
A total of 300 pigs (initial BW = 33 lbs.) were randomly assigned to 3 treatment groups (6 pens/treatment). Group 1 consisted of a positive control and included a complete feed with no mitigants. Group 2 consisted of the positive control + Sal CURB at 6.5 lb./ton. Group 3 consisted of the positive control + CaptiSURE at 20 lb./ton. Sal CURB contains a blend of aqueous formaldehyde and organic acids and is labeled to maintain feed and feed ingredients Salmonella-negative for up to 21 days. CaptiSURE is a liquid energy source from palm kernel oil, containing medium chain fatty acids (MCFA).

Facilities
The study was conducted at a Biosecurity Safety Level-2 facility (Pipestone Applied Research, Pipestone, MN) set up to model a commercial barn. The BSL-2 facility consisted of 6 individual rooms with filtered intake and exhaust air, clean and dirty hallways and bio-secure entry practices between rooms. For this study, 3 rooms were used with each room representing 1 treatment. Pigs were sourced from a herd documented to be free of all 3 viral pathogens used in this study by monthly testing and clinical history. Feeding duration of the study was 15 days.

Virus Challenge
Feed was challenged by creating a 454g "ice block," consisting of 100 mL of PRRSV (5 logs TCID50/mL, Ct = 21.38), 100 mL of PEDV (5 logs TCID50/mL, Ct = 24.25), 100 mL of SVA (5 logs TCID50/mL, Ct = 20.72) and 154 mL of minimal essential medium. Blocks were frozen at -80° C and dropped into each feed bin on days 0 and 6 of the study. The blocks were allowed to melt and augured into the designated room for pigs to consume via natural feeding behavior.

Metrics
Ante-mortem samples, including feed samples and oral fluids, were collected across the 6 pens from each of the 3 rooms at 0, 6 and 15 days post-inoculation (DPI). Post-mortem samples were collected from 30 pigs from each room at 15 DPI. Diagnostics on collected samples included serum for PRRSV, tonsil for SVA and rectal swabs for PEDV. Samples were evaluated for the presence of viral nucleic acid by PCR and nucleic acid sequencing of the ORF 5 was performed on select samples, as needed. In addition, start and end body weights were collected from all pigs (0 and 15 DPI). Differences in growth performance between groups were analyzed for significance ($P < 0.05$) using ANOVA. In addition, pigs were scored daily for the presence of clinical signs, including dyspnea/weight loss/rough hair coat (PRRSV), diarrhea (PEDV) and lameness/vesicles (SVA).
Results
Feed samples indicated the presence of RNA from all viruses regardless of treatment. Similarly, oral fluid samples tested positive for viral RNA regardless of treatment (Table 1). This shows feed in the bins was successfully contaminated using the ice block model, resulting in delivery of all 3 viruses to all rooms. The presence of viral RNA in feed samples treated with formaldehyde or MCFA has previously been documented and does not indicate the presence of infective virus, but rather indicates the presence of RNA.\(^5,6\)

Table 1. Detection of viral RNA in feed & oral fluid samples by PCR.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRRSV</th>
<th>PEDV</th>
<th>SVA</th>
<th>PRRSV</th>
<th>PEDV</th>
<th>SVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>MCFA</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>(+) control</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Pigs in the positive control treatment were infected by all three viruses as determined by diagnostic analysis and observation of clinical signs (Table 2). Pigs in both the Sal CURB and CaptiSURE treatments remained free of viral infection for each of the three viruses. Pigs in the positive control treatment had a reduced average daily gain compared with pigs in the formaldehyde and MCFA treatments.

Table 2. Postmortem sample results and growth performance measures.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRRSV</th>
<th>PEDV</th>
<th>SVA</th>
<th>PRRSV</th>
<th>PEDV</th>
<th>SVA</th>
<th>ADG (lb./d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>2.06(^a)</td>
</tr>
<tr>
<td>MCFA</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>2.09(^a)</td>
</tr>
<tr>
<td>(+) control</td>
<td>100%</td>
<td>13%</td>
<td>43%</td>
<td>100%</td>
<td>17%</td>
<td>100%</td>
<td>1.71(^b)</td>
</tr>
</tbody>
</table>

\(^a, b\) Differences in growth rate determined to be significant at \(P < 0.05\).

1 Represented by virus recovered from animals (PRRSV=serum, PEDV=rectal swabs, or SVA=tonsil)
2 Represented by clinical signs of disease (diarrhea, dyspnea, rough hair, or lameness)

Conclusion
Under the conditions of this study, the “ice block” challenge model successfully infected pigs with all three viruses. This large scale, field-based model demonstrated the risk of contaminated feed as a vehicle for viral transmission to pigs. Both formaldehyde and a medium chain fatty acid blend were effective at preventing transmission of all three viruses through feed in this model. Additionally, performance of pigs in the formaldehyde and medium chain fatty acid blend treatments were significantly improved compared to the positive control pigs. Sal CURB and CaptiSURE may be used as part of a comprehensive biosecurity plan.

References
1. An evaluation of the efficacy of Sal CURB® ASF and CaptiSURE™ at reducing the risk of infection following consumption of feed contaminated with PRRSV 174, PEDV, and SVA, TD-19-5265.