Effect of organic acid blend on feed contaminated with African Swine Fever virus

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Since the African Swine Fever virus (ASF/ASFv) reached China in August of 2018, articles have been published and seminars held on "what to do next" to improve biosecurity in an effort to prevent the spread of the disease. With the absence of an available vaccine, ASF has continued to jump from one farm to another in affected countries. The question seems to be, are we missing something to help manage the spread of ASF or is the spread of ASF beyond our control?

ASF requires stakeholders to perform stringent biosecurity protocols and procedure that were not commonly practiced by most farms in the past. The Food and Agricultural Organization (FAO) along with other institutions provided biosecurity recommendations that may be applied on numerous swine production systems. This includes understanding the routes of disease transmission and its implication in biosecurity. One aspect of biosecurity is that feed can become contaminated and may play role in the spread of viral disease.

For decades, acidifiers or blends of acids have been used in swine to improve growth performance. Specific mode of action has yet to be determined but many producers use acidifiers to reduced stomach pH after weaning when the piglet's digestive system is still immature as it is believed that acidifiers may increase proteolytic enzyme activity in young animals, allowing more protein digestion. An increase in growth could also be caused by organic acids acting as an energy source in the gastrointestinal tract. A study featuring organic acid blend ACTIVATE® DA nutritional feed acid from Novus International, Inc. showed that improved growth performance on post weaning piglets could be due to a reduction of pathogenic bacteria in the gut (Table 1). This anti-bacterial effect was also documented in a trial by orally challenging weanling pigs with *E. coli* K88^{+}. Interestingly, the study showed that the organic acids in ACTIVATE® DA were able to improve performance of piglets and decrease *E. coli* count in the ileum (Table 2). Bacterial growth in the gut can be affected by acidic conditions, however the mode of action seen in inorganic acids is different from that of organic acids like fumaric, benzoic and 2-hydroxy-4-methylthio-butanoic acid (HMTBa). HMTBa is a well-researched organic acid that is converted to methionine once absorbed in the gut.

Unlike hydrochloric acid, organic acids in undissociated form during low pH conditions are lipophilic and can diffuse across the membranes of bacteria, allowing the organic acid inside the bacterial cell and negatively impacting bacterial activity. Hydrogen can be dissociated from organic acids due to the high pH of the cytoplasm in the bacteria, resulting in a reduction of the in pH inside the cell. This scenario disrupts enzymatic functions and nutrient transport systems ultimately leading to bacterial cell death.

Table 1. Effect of antibiotics and organic acids on growth performance of pigs 4 weeks post-weaning

Item	Control	Antibiotic[1]	Potassium Diformate[2]	ACTIVATE DA[2]	SEM	p-value
ADG (g/d)	323[b]	395[a]	354[ab]	371[a]	16.27	0.03
ADFI (g/d)	539	576	542	545	31.33	0.82
Feed:Gain (g/g)	1.68[a]	1.46[b]	1.52[ab]	1.47[b]	0.06	0.04

[a,b] Means in a row without a common superscript differ ($p<0.05$), ADG = average daily gain; ADFI = average daily feed intake.

[1]Antibiotic treatment was fed 200 ppm Chloretetracycline and 60 ppm Lincospectin; Potassium Diformate and ACTIVATE DA at 0.5%
ACTIVATE DA is a feed acidifier composed of fumaric, benzoic and HMTBa (2-hydroxy 4-(methylthio) butanoic acid).
Li et al., 2008 Asian-Aust. J. Anim. Sci 21:252 – 261

Table 2. Effect of feeding antibiotics and organic acids on growth performance and ileum digesta microflora of weaned pigs after *E. coli* K88[+] challenge.

Item	Control	Antibiotic[1]	ACTIVATE DA 0.5%	ACTIVATE DA 1.0%	SEM	p-value
Days 0 – 4 (Pre-challenge)						
ADG (g/d)	221	246	221	205	14.40	0.29
ADFI (g/d)	270	283	279	251	20.49	0.71
Feed:Gain (g/g)	1.21	1.15	1.26	1.24	0.06	0.56
Days 5 – 14 (Post-challenge)						
ADG (g/d	280[a]	341[b]	318[b]	315[b]	11.30	0.01
ADFI (g/d)	432	461	443	435	20.70	0.77
Feed:Gain (g/g)	1.53[a]	1.34[b]	1.39[b]	1.40[b]	0.04	0.03
Days 0 – 14						
ADG (g/d	270	307	290	281	9.22	0.07
ADFI (g/d)	390	406	396	383	16.87	0.79
Feed:Gain (g/g)	1.44	1.32	1.36	1.36	0.04	0.26
Ileum microflora (d 14)						
Lactobacilli	6.89	7.05	7.00	7.07	0.05	0.08
E. coli	4.04[b]	3.91[a]	3.93[a]	3.93[a]	0.04	0.08

[a,b] Means in a row without a common superscript differ ($p<0.05$), ADG = average daily gain; ADFI = average daily feed intake.
[1]Antibiotic treatment was fed 100 ppm Colistin sulfate, 50 ppm Kitasamycin and 50 ppm Olaquindox.
Li et al., 2008 Asian-Aust. J. Anim. Sci 21:252 – 261

Viral Contamination in the Feed

A research study conducted by Pipestone in the U.S. (Dee et al., 2018) concluded that viruses like porcine epidemic diarrhea virus (PEDv) can survive in feed. A study by Trudeau et al., 2016 found that ACTIVATE® DA can effectively reduce the survivability of PEDv and reduce the risk of disease spread. This study inspired researchers to see if the same was true with ASFv. With the objective of improving feed biosecurity, Novus conducted a trial at Key Laboratory of Veterinary Biotechnology at Vietnam National University of Agriculture in Vietnam to evaluate the efficacy of ACTIVATE® DA against ASFv in feed and to determine what amount of the feed additive would be needed to reduce the survival rate of the virus in contaminated feed.

Trial Design

For the trial, pig starter mash feed without antibiotic, formaldehyde or organic acids was obtained and screened by RT-PCR to ensure an ASFv-negative status prior to use. The organic acid blend ACTIVATE® DA was prepared at 0.2% and 0.5% inclusion. Feed samples were spiked with a solution containing ASFv (VNUA/HY-ASF1/Vietnam/2019) that had a viral concentration of 10^6 $TCID_{50}$/mL, including the positive control feed samples (Table 3). All samples were incubated at room temperature of around 20°C and 60% relative humidity during the season that the experiment was performed. Samples were collected at three independent times; 1, 3 and 7 days post inoculation (DPI) and collected samples were used to inoculate PAMs (cells obtained from pig lungs, also known

as porcine alveolar macrophages) for virus isolation. The cell supernatants were then collected and assessed using RT-PCR for viral gene copies and HAD assay for infectivity of ASF.

Table 3. Treatment Design and Contamination

Treatment	Additive Supplementation	ASFv (10^6)
Positive Control	No	Yes
Treatment 1	0.2% ACTIVATE DA	Yes
Treatment 2	0.5% ACTIVATE DA	Yes

Assessment of ASFv virulence by RT-PCR

Results of **RT-PCR** showed that ACTIVATE® DA at 0.2% and 0.5% reduced the genetic material of ASFv in feed on day 1 with higher Ct values compared to the positive control indicating moderate amount of ASFv nucleic acids (Figure 1). A similar trend was observed on day 3, while on day 7 the Ct value of the treatment groups showed weak reactions compared to the control group. It was observed through Ct values that the organic acid blend had a rapid action in reducing the total genetic material of ASFv in feed.

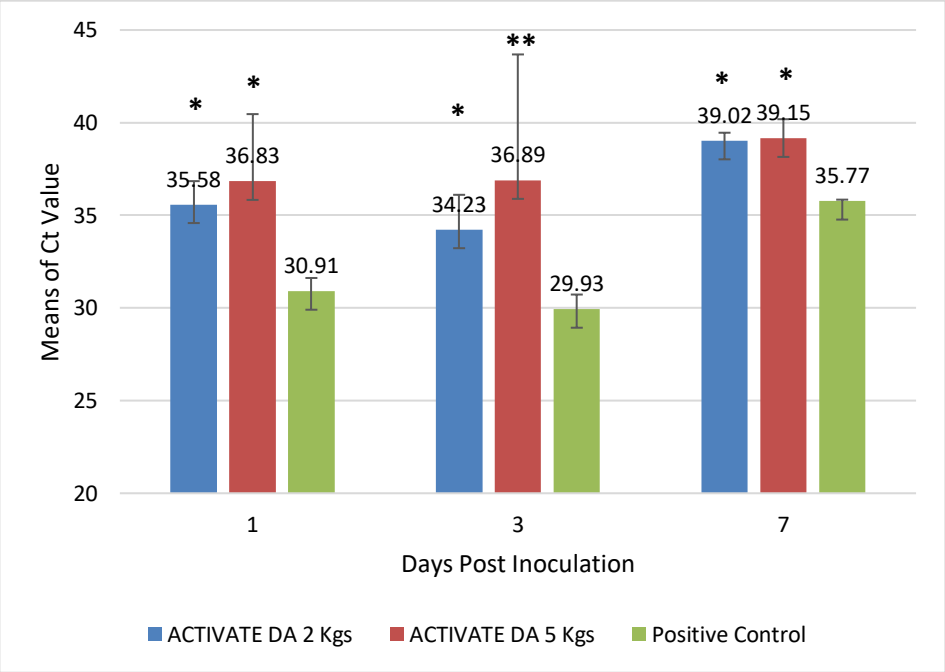

Figure1. ASFv virulence by RT-PCR. Ct < 29 are strong positive reactions indicative of abundant target nucleic acid in the sample. Ct of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid. Ct of 38-40 are weak reactions indicative.

Assessment of ASFv virulence by HAD_{50}

The majority of the ASFv strains produced the haemadsorption reaction (HAD) due to adsorption of pig red blood cells on virus-infected leukocytes. The importance of this test relies on its specificity: no other pig viruses are capable of creating HAD in leukocyte cultures. Consistent with the RT-PCR results, ASFv titration assay showed that ACTIVATE® DA had significant reduction activity against ASFv in feed from day 1 to 7. Furthermore, at 0.2% inclusion the nutritional acid blend was shown to reduce survivability of the virus in feed by 99% while 0.5% inclusion showed

References available on request. Details first appeared in International Pig Topics, vol. 35 no. 8.

99.9% within day 3. This may indicate that higher concentration of acid had better capability of decreasing viral load in the feed. At day 7, a 1 log reduction on means of the positive control was observed, which can be due to the half-life of the virus; its ability to survive in feed may have been affected by the virus's specific structural characteristics (Figure 2). However, 2 logs reduction of ACTIVATE® DA at both inclusions was observed at day 7 compared to the control, showing efficacy against ASFv in feed.

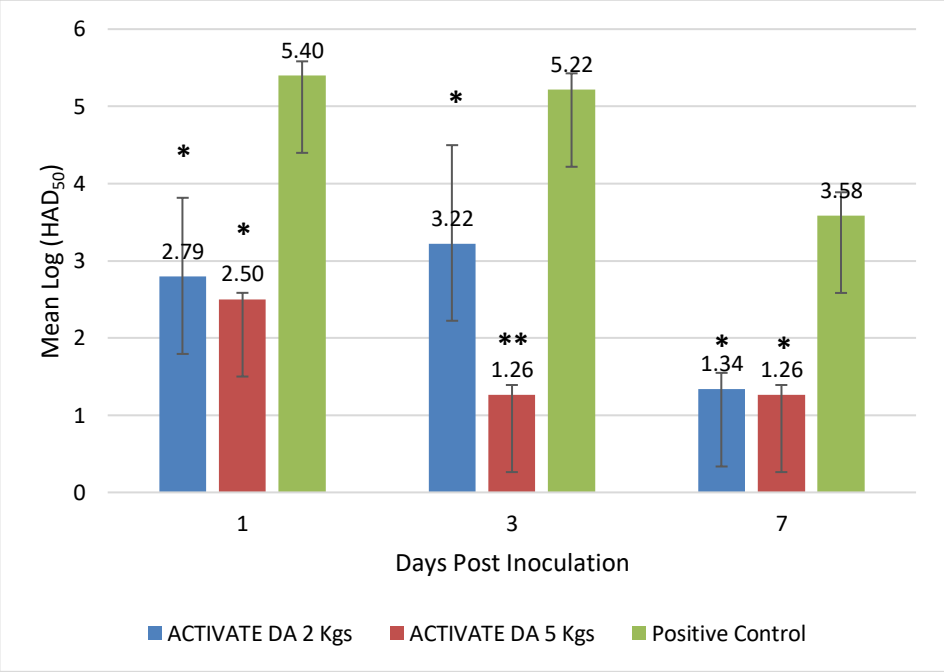

Figure 2. ASFv virulence by HAD50. Virus reduction is expressed on a logarithmic scale. A log reduction takes the power in the opposite direction wherein a log reduction of 1 is equivalent to a 10-fold reduction.

Summary

Key ingredients in ACTIVATE® DA showed a strong synergistic effect to help reduce the survivability of ASFv in feed and demonstrated rapid viricidal reaction. In this trial, it can be concluded that both 0.2% (2 kg/ton) and 0.5% (5kg/ton) inclusion of ACTIVATE® DA can reduce the risk of ASFv transmission through feed contamination.

Blends of organic acids were designed to be effective against bacteria and for feed preservation. Recent findings suggest that organic acids can help mitigate viral contamination in feed. Considering the positive effects on pig gut health and performance, an organic acid blend with a shown anti-viral effect in feed can maximize the return for swine operations.

As ASF continues to spread, the need to review all angles is a must. Feed biosecurity is only a fraction of a holistic approach to reduce the risk of disease. It must be partnered with cleaning/disinfection practices of buildings and vehicles, controlling the movement of people, pest and other animals on the farm, sourcing good replacement stock that is free of diseases, and other practical procedures. Farmers, service providers and supply chain in pig and feed production should live a culture that advocates strong biosecurity so that ASFv spread can be within their control.

References available on request. Details first appeared in International Pig Topics, vol. 35 no. 8.