SWINE HEALTH INFORMATION CENTER
INTERIM RESEARCH GRANT REPORT FORMAT

I. Project Title: Stability of Senecavirus A in animal feed ingredients (SHIC 18-211)

Principal Investigator: Diego G. Diel

Institution: SDSU

Date Report Submitted: 03/26/19

II. Industry Summary

This study evaluated the stability of Senecavirus A, a picornavirus surrogate for foot-and-mouth disease virus (FMDV), and a pathogen that is known to survive for prolonged time in several swine feed ingredients. Common swine feed ingredients including conventional soybean meal (SBM-C), DDGS, lysine and Vitamin D were inoculated with a constant dose of SVA and incubated under different temperatures (4ºC [39.6ºF], 15ºC [59ºF] and 30ºC [86ºF]) to assess the effect of temperature on the stability of the virus. Samples incubated at each temperature were collected weekly for 14 weeks (days 1 through 91) and the amount of viable SVA was determined by virus titrations in the laboratory. Control samples consisted of stock virus incubated in a plastic container without a feed ingredient. The control samples were included in all temperatures tested, collected and processed following the same sample schedule as above. SVA was inactivated within 7-14 days when incubated at 30ºC (86ºF). Lower incubation temperatures (4ºC [39.6ºF], 15ºC [59ºF]), however, favored survival of SVA for 28 or up to 91 days, respectively. The results from this study demonstrate that SBM and DDGS provide a good matrix for the survival of SVA. Lysine and vitamin D, on the other hand only supported SVA survival for 21 days, even when incubated at lower more favorable temperatures (4ºC [39.6ºF]). A clear effect of temperature on the stability of the SVA was also observed. When SVA spiked-SBM or -DDGS were incubated at 4ºC, infectious SVA was recovered from these samples until the end of the experiment on week 14 or day 91 post-incubation. It is important to point out that SVA viability decayed much faster (7-21 days) in control samples, in which the virus stock was deposited directly in a plastic tube without a feed matrix. The half-life, or the time required for infectious SVA amounts to decrease by one-half, were also

determined in all feed ingredients. These results, consistent with the decay rate, show an extended half-life for SVA in SBM and DDGS when incubated at 4ºC (10.9 and 37.9 days, respectively). Incubation at higher temperatures results in rapid degradation of the virus and very short half-life's (1.25 and 1.36 days for SBM and DDGS, for example). In conclusion, results from these studies confirm that common swine feed ingredients such as SBM and DDGS provide a good environment for virus survival, increasing the overall stability of SVA, an important swine pathogen and surrogate for FMDV to survive for long periods of time. A clear effect of temperature was observed, with higher environmental temperatures resulting in rapid virus decay even in the most favorable feed ingredients. These results may help the swine industry to devise mitigation strategies that consider holding times for feed ingredients that are imported from countries where foreign animal diseases are endemic.

III. Stated Objectives from original proposal

The objective of this study was to assess the stability of SVA in feed ingredients. The specific aims related to this objective are:

Specific aim 1: To determine the rate of virus decay as a function of time and temperature; and

Specific aim 2: To estimate the half-life of SVA in high risk feed ingredients

Successful completion of the proposed study will provide critical information on the stability and rate of decay of SVA in feed. This information can be used to estimate required holding times for feed ingredients that would reduce or minimize the risk of pathogen transmission through feed.

IV. Progress toward meeting objectives

Objective 1 is progressing as planned. We have spiked high risk feed ingredients including SBM, DDGS, Lysine and Vit D with a constant dose of SVA (10^5 $TCID_{50}$) and

incubated the virus-ingredient mixtures at different temperatures (4, 15 and 30°C). Virus titers were determined at different intervals post-inoculation.

Objective 2 is progressing as planned. SVA T1/2 calculations were performed with titers obtained at the different incubation temperatures. A few Vit D and Lysine samples are being re-titrated.

V. Status of project regarding stated timeline

Overall the project is progressing as planned. Virus inoculations and titrations have been complete and T1/2 calculations were performed.

VI. Modifications of project from original proposal

The design of the original proposal has not been changed. Only one change in the number of experiments was made. We decided to perform 3 independent experiments on each temperature to increase confidence in our results.

VII. Results

Virus decay over time. Virus infectivity and the amount of infectious SVA following incubation of SVA-spiked SBM, DDGS, Lysine and Vit D at 4, 15 or 30°C were determined using microtitration assays. Table 1 presents the summary of the sampling schedule and timeline used on the study. Duplicate samples were collected on each time point, resuspended in 15 ml of PBS and cleared by centrifugation. Cleared supernatants were serially diluted (10-fold) in RPMI and each dilution inoculated into four wells of a 96-well plate. H1299-cells suspension were added to each well and plates incubated at 37°C for 48 hours. After 48h incubation, cells will be fixed with 80% aqueous acetone solution and stained with anti-SVA VP2-fluoresent isothiocyanate-conjugated monoclonal antibody (SD214-188). Infectious SVA titers were determined using the

Spearman-Karber method and expressed as $TCID_{50}$/ml. The titer on each sample was corrected to the volume of PBS used to reconstitute the feed ingredient. This was done by multiplying the viral titer by 15.

Table 1 – Sampling schedule/timetable

Temp	Sample collection points/Days													
	1	7	14	21	28	35	42	49	56	63	70	77	84	91
4°C	x	x	x	x	x	x	x	x	x	x	x	x	x	x
25°C	x	x	x	x	x	x	x	x	x	x	x	x	x	x
30°C	x	x	x	x	x	x	x	x	x	x	x	x	x	x

As shown in **Fig. 1**, marked differences in titers were observed between the three test temperatures. Importantly, incubation SVA-spiked feed ingredients at the higher temperatures (15 and 30°C) lead to rapid virus decay with no infectious virus being detected after day 14 or 28 post-incubation, respectively (Fig. 1B and C). Incubation at 30°C led to rapid virus decay even in ingredients like SBM and DDGS which seem to provide a very good matrix for SVA survival at low temperatures (4°C), as evidenced by detection of infectious SVA in those ingredients incubated at 4°C up to day 91 post-incubation. It is important to note that no infectious SVA was detected in control samples (no feed matrix) passed day 21 post-incubation even at low incubation temperatures (4°C). These results demonstrate that the matrix provided by specific feed ingredients provides a very good environment for survival of viral pathogens for extended periods of time.

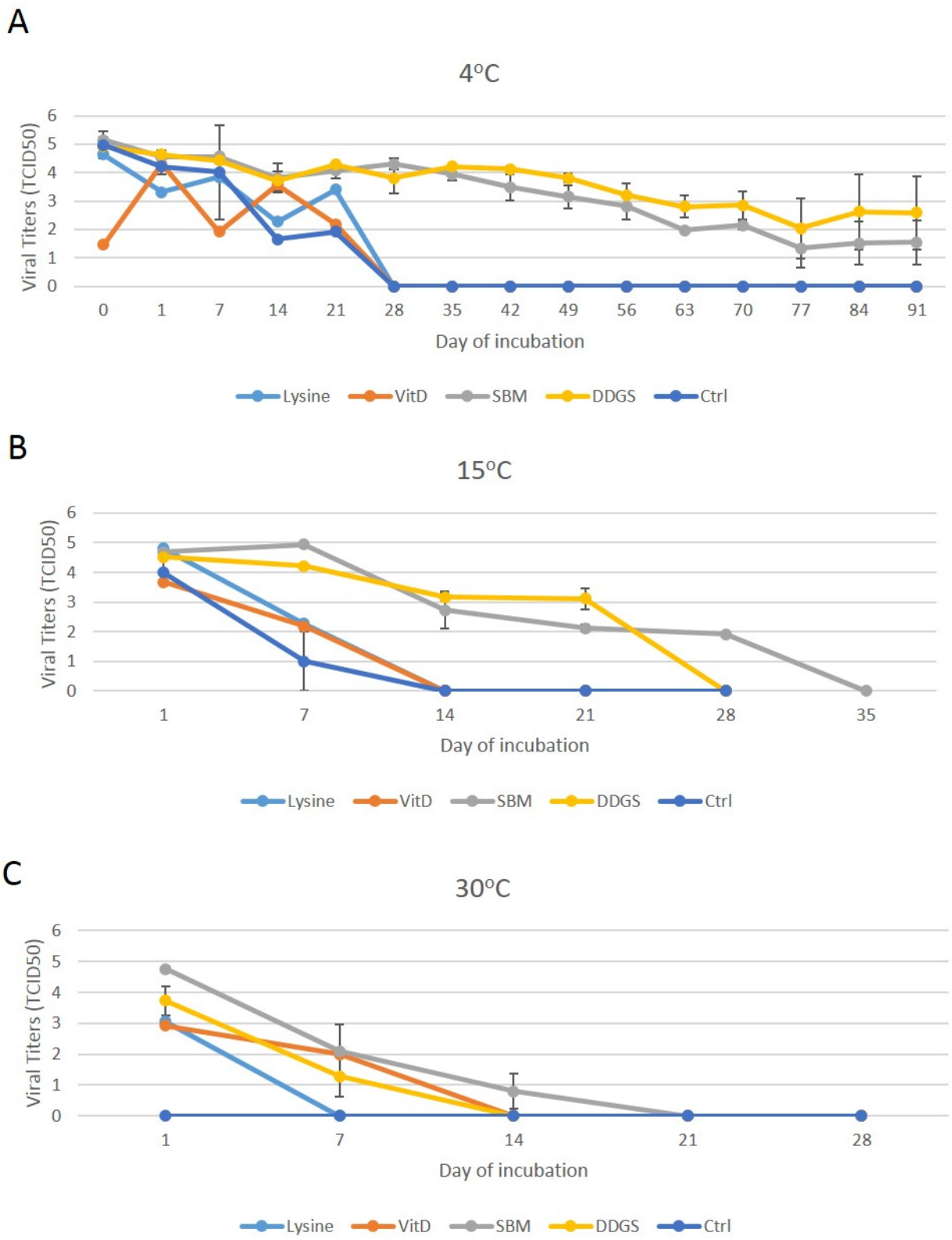

Fig. 1 Stability of Senecavirus A in animal feed ingredients under different temperatures. Five grams of each feed ingredient were inoculated with 10^5 $TCID_{50}$ of SVA, incubated at 4, 15 or 30°C and processed for microtitration assays. SVA titers are expressed as log10 median tissue culture infectious dose 50 ($TCID_{50}$)

Half-life calculations. Half-life estimates were calculated for four SVA at the four ingredients and three treatment temperatures (Table 2). Overall, half-life appeared to be influenced by ingredient type and temperature, DDGS and SBM incubated at 4°C displaying the longest half-lives, 37.9 days and 13.9 days, respectively. SVA half-lives at higher temperatures were much shorter (Table 2). Most importantly, presence of the feed matrix, especially of SBM and DDGS, appeared to extend the half-life of the virus.

Table 2 – Half-life estimates for SVA in different feed ingredients incubated at different temperatures.

Treatment	slope	half life	sd of half life	lower CI	upper CI
SBM 4	-0.06334	10.94	1.19	8.55	13.33
SBM 15	-0.19608	3.53	0.60	2.28	4.78
SBM 30	-0.50699	1.36	0.29	0.69	2.04
DDGS 4	-0.01826	37.95	8.58	20.75	55.16
DDGS 15	-0.05301	13.07	5.55	1.55	24.59
DDGS 30	-0.55333	1.25	0.52	0	2.92
Vit. D 4	-0.27087	2.55	0.83	0.64	4.47
Vit. D 15	-0.58123	1.19	0.86	0	3.60
Vit. D 30	-0.63719	1.08	0.72	0	3.09
Lysine 4	-0.13045	5.31	4.11	0	14.80
Lysine 15	-0.95549	0.72	0.06	0.54	0.90
Lysine 30	-5.75376	0.12	0.01	0.07	0.16
Control 4	-0.29441	2.35	0.22	1.83	2.87
Control 15	-0.96453	0.71	0.03	0.62	0.81
Control 30	-6.84676	0.10	0.005	0.07	0.12

[a]Half-life calculations were performed using the viral titers detected throughout the timeline depicted in Table 1 and Fig. 1. 95% Wald confidence interval.