PROGRESS PROJECT REPORT

REPORT SUBMITTED TO

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PROJECT TITLE

Studies on Control Measures for Avian Reovirus Variants and Other Enteric Viruses in Poultry Flocks

INSTITUTION

The Pennsylvania State University,
College of Agriculture Sciences
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PERIOD OF THIS REPORT

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A. Project Objectives

This project aims to achieve two major objectives as:

1. Evaluate efficacy of non-metallic or "soft" disinfectants for prevention of avian reovirus (ARV) and other enteric virus infections in broiler and layer production flocks.

2. Develop more effective control measures and strategies for better control and prevention of ARV variants and other enteric viruses in poultry flocks.

B. Progress Results

Progress results have been made towards objectives during this research period. Two non-metallic or "soft" disinfectant products of Shield Plus (Timac Agro, USA, www.us.timacagro.com), Assist NPS's solution (Assist Natural Products and Services, LLC, www.assist-nps.com), and others were obtained to conduct virus inactivation tests in laboratory conditions. Test results are summarized as the followings.

1. Test procedures of the Shield Plus disinfectant for virus inactivation tests

1) Preparation of "Shield Plus" powder (SPP) for different % concentrations were prepared as the Table-1.

Table-1. Preparation of Shield Plus" powder (SPP) in different concentrations (%) for inactivation tests on avian viruses (e.g., ARV, FAV, IBDV, IBV)

	1%	2%	3%	4%	5%	6%	8%	10%	15%	20%
SPP	0.25g	0.5g	0.75g	1.0g	1.25g	1.5g	2.0g	2.5g	3.25g	5g
virus	24.75ml	24.5ml	24.25ml	24ml	23.75ml	23.5ml	23ml	22.5ml	21.75ml	20ml
total	25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml

Note: (1) Firstly, weight the SPP and place into 50ml centrifuge tube; (2) Secondary, add the 1:10 diluted stock virus into each of the 50ml centrifuge tubes containing SPP.

2) Because the SPP will start reaction when it mixes with liquid/water, so do not add the test virus to the tubes until an incubation time count is ready.

3) The 50 ml tubes containing the 25 ml of SPP and virus mix need to keep mixing (place on a shaker plate) during the entire incubation time at room temperature.

4) Watch the incubation time, take supernatant samples from the reaction tubes at 5 min, 10 min, 15 min, and 30 min to inoculate LMH cells, inoculate 4 wells per sample in a 24-well cell culture plate, 0.1 ml inoculum per well.

5) The inoculated cell culture plates are incubated at 37C/CO2 incubator for 30 min, then 1.0 ml of 2% FBS maintenance medium is added to each well, the plates are placed in 37C/CO2 incubator.

6) Daily observation of the inoculated LMH cells, record the occurrence of viral cytopathic effect (CPE) cells.

7) The inoculated LMH cell cultures are terminated after 6 days post incubation.

8) If no CPE observed, the inoculated LMH cells are harvested for a secondary cell passage.

9) Examine the 2nd cell pass as the above 7) - 9) steps.

2. Shield Plus disinfectant for virus inactivation tests on avian reovirus (ARV) and fowl adenovirus (FAV)

The "Shield Plus" powder product was prepared in serial concentrations of 5%, 8%, 10%, 15% and 20% in cell culture medium for Avian Reovirus (ARV) and Fowl Adenovirus type 1 (FAV-1) inactivation studies using LMH cell cultures.

Three ARV strains (S1133 vaccine strain, Reo/PA/Layer/29730/11 (genotype 2), , and three FAV-1 strains (CELO, KR5, C229) were newly propagated for this study. Each of these virus strains was tested in each concentration of the Shield Plus for 5 min and 15 min reaction times, respectively.

The Shield Plus powder disinfectant effectively inactivated or killed ARV and FAV-1 strains at 8%, 10%, 15% and 20% concentrations when dissolved in PBS or water within 5 or15 min reaction time in a laboratory condition (Table 2).

Table 2. Results of Shield Plus disinfectant for inactivation tests on Avian Reovirus (ARV), Fowl Adenovirus type 1 (FAV-1: CELO, KR5 and C229 strains).

Avian Virus	Concentration (%) of Shield Plus on avian virus inactivation tests									
	5%		8%		10%		15%		20%	
	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m
ARV (S1133)	0%	50%	100%	100%	100%	100%	100%	100%	100%	100%
AIV (PA29730/11)	0%	50%	100%	100%	100%	100%	100%	100%	100%	100%
ARV (PAxxxxx/xx)	0%	50%	100%	100%	100%	100%	100%	100%	100%	100%
FAV-1 (CELO)	0%	50%	100%	100%	100%	100%	100%	100%	100%	100%
FAV-1 (KR5)	0%	25%	100%	100%	100%	100%	100%	100%	100%	100%
FAV-1 (C229)	0%	25%	100%	100%	100%	100%	100%	100%	100%	100%

Note: 100% = 100% virus inactivation results; 0% = the virus was not inactivated.

3. Shield Plus disinfectant for virus inactivation tests on infectious bursa disease virus (IBDV)

1) The Shield Plus powder at 8% and 10% concentrations inactivated most (>90%) IBDV after 10-30 min reaction time, since no viral cytopathic effects (CPE) cells were seen during the observation period of 6 days post inoculation; however, some residual virus (not killed) was amplified at the second cell passage in causing CPE cells at 4-6 days pi.

2) The Shield Plus powder at 3% to 6% concentrations could kill at least 50% of the virus within 10-30 min, since no CPEs were seen in the first 3 days pi.

3) The Shield Plus powder at 1% and 2% concentrations appeared little effect on IBDV inactivation in 10-30 min reaction time.

4) The effectiveness of the "Shield Plus" powder disinfectant on virus inactivation could be increased along with increasing the reaction time. We plan to extend reaction times of the virus inactivation results in next study period.

5) A Table Summary of Results (Table-3).

Table-3: Results "Shield Plus" Powder Disinfectant on Infectious Bursa Disease Virus (IBDV) Inactivation Studies

Shield Plus (%)	Reaction time with IBDV	1st pass 3 dpi	1st pass 6 dpi	2nd pass 3 dpi	2nd pass 6 dpi	Result of % effective
1%	10min	2(±)/2	2+/2	Because the 1st cell passage showed IBDV was not completely inactivated, thus there was no need for the 2nd cell passage to test.		~ >20%
	15min	2(±)/2	2+/2			~ >20%
	30min	2(±)/2	2+/2			~ >20%
2%	10min	2(±)/2	2+/2			~ >20%
	15min	2(±)/2	2+/2			~ >20%
	30min	2(±)/2	2+/2			~ >20%
3%	10min	0/4	4+/4			~ >50%
	15min	0/4	4+/4			~ >50%
	30min	0/4	4+/4			~ >50%
0%, BDV+	30min	4+/4	4+/4	4+/4	4+/4	(+) control
0%, PBS	30min	0/4	0/4	0/4	0/4	(-) control
4%	10min	0/4	4+/4	Because the 1st cell passage showed IBDV was not completely inactivated, thus there was no need for the 2nd cell passage to test.		~ >50%
	15min	0/4	4+/4			~ >50%
	30min	0/4	4+/4			~ >50%
5%	10min	0/4	4+/4			~ >50%
	15min	0/4	4+/4			~ >50%
	30min	0/4	4+/4			~ >50%
6%	10min	0/4	4+/4			~ >50%
	15min	0/4	4+/4			~ >50%
	30min	0/4	4+/4			~ >50%
8%	10min	0/4	0/4	0/4	4+/4	~ >90%
	15min	0/4	0/4	0/4	4+/4	~ >90%
	30min	0/4	0/4	0/4	4+/4	~ >90%
10%	10min	0/4	0/4	0/4	4+/4	~ >90%
	15min	0/4	0/4	0/4	4+/4	~ >90%
15%	10min	0/4	0/4	0/4	0/4	100%
	15min	0/4	0/4	0/4	0/4	100%
20%	10min	0/4	0/4	0/4	0/4	100%
	15min	0/4	0/4	0/4	0/4	100%
0%, BDV+	15min	4+/4	4+/4	4+/4	4+/4	(+) control
0%, PBS	15min	0/4	0/4	0/4	0/4	(-) control

Note: (1) 100% = 100% virus inactivation results; 0% = the virus was not inactivated.

4. Summary of the Assist NPS's solution test results

1) Preparation of the Assist NPS's Solution

The stock solution at 5000 PPM concentration was provided by the Assist Natural Products and Services LLC.

A serial of dilutions was prepared in deionized water (dH_2O), the solution and dH_2O ratios were made to yield serial PPM concentrations in the solution, see Table 4-1.

Table 4-1. Preparation of serial PPM concentrations of the Assist NPS's stock 5000 solution

Volume of stock solution (ml)	Volume of dH_2O diluent (ml)	Total volume (ml)	The solution concentration (PPM)
12.8	0	12.8	5000
10.0	2.8	12.8	4000
7.5	5.3	12.8	3000
5.0	7.8	12.8	2000
2.5	10.3	12.8	1000
1.8	11.0	12.8	700
1.3	11.5	12.8	500
1.0	11.8	12.8	400
0.75	12.05	12.8	300
0.5	12.3	12.8	200
0.25	12.55	12.8	100
0.125	12.675	12.8	50
0.025	12.775	12.8	10

2) Safety test of the Assist NPS's solution to chicken embryos

The Assist NPS's solution at a concentration of 1000 PPM is safe to ≥9-day-old embryonating chicken eggs (ECEs) at 0.2ml/ECE, and at the stock solution concentration of 5000 PPM is safe to ≥15-day-old ECEs at 0.2ml/ECE (Table 4-2).

3) Effects of the Assist NPS's Solution on avian influenza virus (AIV) inactivation

The Assist NPS's solution at 400-500 PPM effectively inactivated AIV subtypes H1N1, H3N2, H5N2 and N7N2 in 5 min tested in lab condition (Table 4-3).

4) Effects of the Assist NPS's Solution on infectious bronchitis virus (IBV) and Newcastle disease virus (NDV) inactivation.

The Assist NPS's solution in 300-500 PPM effectively inactivated or killed both IBV and

NDV in 5 min tested in lab condition (Table 4-4).

Table 4-2. Safety test of the Assist NPS's Solution on chicken embryo's tolerance

PPM concentration in the solution	9-day-old chicken embryo		15-day-old chicken embryo	
	dead/total	death rate	dead/total	death rate
5000	5/5	100%	0/5	0%
4000	5/5	100%	0/5	0%
3000	1/5	20%	0/5	0%
2000	2/5	40%	0/5	0%
1000	0/5	0%	0/5	0%
700	0/5	0%	0/5	0%
H_2O	0/5	0%	0/5	0%

Note: 100% = virus was completely inactivated; 0% = virus was not inactivated.

Table 4-3. The Assist NPS's Solution on AIV inactivation results

PPM concentration in the solution	H1N1		H3N2		H5N2		H7N2	
	5min	10min	5min	10min	5min	30min	5min	10min
500	100%	100%	100%	100%	100%	100%	100%	100%
400	100%	100%	100%	100%	100%	100%	100%	100%
300	80%	80%	100%	100%	100%	100%	60%	80%
200	60%	60%	100%	100%	100%	100%	0%	20%
100	20%	20%	20%	20%	0%	20%	0%	0%
50	0%	0%	0%	0%	0%	0%	0%	0%
10	0%	0%	0%	0%	0%	0%	0%	0%
H_2O	0%	0%	0%	0%	0%	0%	0%	0%

Note: 100% = virus was completely inactivated; 0% = virus was not inactivated.

Table 4-4. The Assist NPS's Solution on IBV and NDV inactivation results

PPM concentration in the solution	IBV-Mass		NDV	
	5min	10min	5min	10min
500	100%	100%	100%	100%
400	100%	100%	100%	100%
300	100%	100%	100%	100%
200	0%	0%	0%	0%
100	0%	0%	0%	0%
50	0%	0%	0%	0%
10	0%	0%	0%	0%
H_2O	0%	0%	0%	0%

Note: 100% = virus was completely inactivated; 0% = virus was not inactivated.

C. Work Plan in Next Research Period

Based on our research findings of the Shiel Plus and Assist NPS's Solution on avian virus inactivation tests, we plan to test the efficacy of these soft disinfectants for protection or reduction of virus infections in experimental chickens, so as to provide guidelines to apply these soft disinfectants in broiler and layer production flocks, and effectively prevent poultry flocks from virus infections and viral diseases.

Copy/paste email from www@soybeanresearchdata.com
Soybean Research Data www@soybeanresearchdata.com
Mon 3/29/2021 9:00 AM

Hello Huaguang Lu,

A Progress Report for the National Soybean Checkoff Database is due on March 31, 2021. Updating your project "Studies on Control Measures for Avian Reovirus Variants and other Enteric Viruses in Poultry Flocks" for 2020 is simple to do and won't take a lot of time! Simply log on to the link provided: https://www.soybeanresearchdata.com/admin/Project.aspx?id=53758

Edit your project to update the progress report.
Reminder: Until this is completed, you will get a notification before the progress report is due in increments of 2-weeks, 1-week, 3-days and the day it is due. After that, you will be contacted directly.

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Researchers: When semi-annual reports are due, they should be relatively brief and higher level. Please include brief statements of goals/objectives, deliverables, milestones and key performance indicators with brief summaries, including some data as appropriate, of progress, accomplishments and deliverables to-date. If the project has been renewed for another year of research or if you have received a 3-month no-cost extension, you may submit an update that is similar to a semi-annual report. For final reports, please complete the template with more detailed descriptions of project goal, objective, deliverable and key performance indicators. Then, in the template, present more detailed project results, deliverables, findings, presentations, publication and meeting lists, key benefits to farmers and other researchers, etc. If necessary, please attached more details, such as tables, figures, detailed results descriptions, publications, etc.

Thank you!

CC: Jennifer Reed-HarryCC: Karen Deimler