FINAL REPORT
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VALIDATION OF THERMAL DESTRUCTION OF AVIAN INFLUENZA VIRUS IN RENDERED ANIMAL PRODUCTS

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Project Summary:

In order to prove that thermal processing of rendered materials will destroy the avian influenza virus, it was first necessary to determine a method to identify presence/absence of the virus. In the initial portion of the study, Dr. Scott developed an enzyme linked immunosorbent assay (ELISA) using the associated hemagglutin and neuraminidase peptides that are found on the exterior of the virus particle. Upon testing this with the purified virus proteins, the test accurately detected the virus. However, upon adding the virus proteins to rendering materials, the high fat matrix interfered with the binding of the antibodies to the proteins and resulted in false test results. Therefore, a polymerase chain reaction (PCR) assay was next tested. Dr. Leaphart conducted trials and determined that the type A influenza virus could be detected in the rendering materials. A thermal trial was conducted and results are pending.

Objective (s):

The objectives of this study are:

1) to develop methods of detecting avian influenza virus within rendering materials using PCR and ELISA technology;
2) to conduct a study on thermally processed rendering materials to determine the effectiveness of the rendering process to destroy the avian influenza virus;
3) to publish data and make recommendations concerning rendering as a method of safely destroying the avian influenza virus.

Project Overview:

The project initiated with work on creating a sandwich/capture ELISA using the same antibody conjugated with a probe. Unless cross-reactivity occurred, this should have been a straight forward project. Reagents were obtained through the Clemson University Diagnostic Laboratory from the National Veterinary lab for detection of avian influenza serotypes sharing the same hemagglutinin (H5) and neuraminidase (N1) antigens found on the most infectious form of the virus. These reagents (antiserum, positive antigen control, weak positive antigen control, and negative control) are routinely used in the field to detect the presence of AI in chicken serum. The reagents have been developed for use in a standard immunodiffusion procedure in agar. In order to have a quicker and more sensitive assay system for use of these reagents, an ELISA procedure was developed at Clemson to detect the AI antigens.

Direct ELISA methods allow for semi-quantitative measurement of antigens whereas capture methods allow quantitative measurements. In the initial stages of the project, a test method to detect AI proteins by ELISA was devised by Dr. Thomas R. Scott. A polyclonal antibody against the proteins on the H5N1 virus coat proteins was obtained. This polyclonal was purified using a T-gel column and the recovered immunoglobulin concentration was determined using a micro-Bradford assay. The antibody was used to coat the plates (first part of the “sandwich). The antigen was allowed to attach to the bound antibody on the plate (the center part of the sandwich). And finally, the antibody was conjugated using horseradish peroxidase (HRP) for detection and this was allowed to bind to the antigen fixed to the plate (last part of the sandwich). Fluorescence levels were measured and compared to a standard curve for known antigen levels.

Dr. Scott measured protein concentrations, and performed SDS-PAGE, Western blots, and checkerboard ELISA with the AI reagents. He determined in early trials that the protein concentrations of the reagents were:

Antiserum = 35.6 mg/ml
Strong Positive reference serum = 24.1 mg/ml
Negative reference serum = 103.3 mg/ml
AI antigen = 34.4 mg/ml

Using SDS-PAGE (12% gel), Dr. Scott was able to detect prominent bands for serum; i.e., albumin and gamma globulins (light and heavy chains). He also detected other minor bands at low and high MW. The AI antigen reveals major bands were mostly between 25 - 75 kDa, but there are others >75 kDa.

On a Western immunoblot of each serum source against the AI antigen, Dr. Scott noted very faint staining with the Negative reference serum. Both the antiserum and the strong positive gave nearly identical Westerns against the antigen protein bands. There was strong staining against bands at 25, 50 and 75 kDa. There were also four visible bands noted at ~80, 120, 150 and 250 kDa.

Dr. Scott then performed checkerboard ELISAs on two different days to test for antiserum dilution against antigen concentrations. Through checkerboard analysis, varying concentrations of the antigen controls were coated on standard 96-well ELISA plates and then blocked with a bovine serum albumin solution. The perpendicular application of varying dilutions of the antiserum was then done with 1 hour incubation. The plates were then washed and a second antibody specific for chicken immunoglobulins was added to all wells at the same dilution. This second antibody is conjugated with horseradish peroxidase (HRP). Following a 30 minute incubation, the plates are again washed to remove any unbound antibodies, and a chromogenic substrate, which provided a color reaction in each well. The optical density (OD) of the wells was read on a plate reader and the OD's were recorded for each antigen-antiserum combination. This chromogenic assay approach provided sensitivity of antigen detection at 0.5-1.0 □g AI antigen at an antiserum dilution of 1:100.

A subsequent checkerboard assay was conducted with the same antigen and antiserum scheme, but a luminescent substrate that is reactive with horseradish peroxidase (HRP) on the second antibody was used. This substrate allows for greater sensitivity of detection of the AI antigens on bound to the plate. The assay results from this assay approach provided sensitivity of detection of the AI antigen at 30-60 ng with the effective antiserum dilution at a range of 1:5000-1:10,000.

Plates were coated overnight at 4C with antigen diluted in calcium carbonate buffer. On the second day, wells were blocked with PBS/1% BSA before antiserum incubations. In the first set of plates, Dr. Scott used both the Antiserum and the Strong Positive sera on respective plates. He began the sera dilutions at 1:100 and went as far as 1:102,400. The wells were coated with 156.25 ng to 1 ug of diluted antigen. It took about 45 minutes to get maximum color change and the readings were nearly the same for both serum sources. The readings were skewed to the

1:100 dilution of sera. A second checkerboard was performed the following day with the AI antiserum alone. The serum dilutions were 1:2 to 1:2048. The plates were read at both 20 and 40 minutes. At both times, the best readings relative to zero controls were ~0.5-1 ug antigen with ~1:100 dilution of antiserum.

Dr. Scott further modified the ELISA for Avian Influenza antigens to work in a chemiluminescence system. The previous assay was conducted with a chromogenic substrate (ABTS). He was able to determine that the chromogenic assay could be run with approximately 1:100 dilution of the antiserum and detection of about 0.5 micrograms of the AI antigen. With the chemiluminescence assay, he is able to use the same AI antisera and second antibody (rabbit anti-chicken Ig-HRP) but use luminol reagent as substrate. The assay detects 30-60 nanograms of AI antigen at about 1:5000 dilution of antisera.

The assay system for detecting AI antigens was now established and would be used for detection of AI antigens in render samples processed in a controlled laboratory setting.

Samples of cooked rendering materials were obtained from the end of the Dupps Supercooker at Valley Proteins, Inc, Ward, SC. The materials were transported to the laboratory and stored under refrigeration until needed. Materials were blended in a commercial food processor to reduce particle size.

AI antigen was added to the rendering materials and Dr. Scott proceeded to test for the ability to detect the antigen in the materials using the tests developed above. Samples were minced and homogenized. Buffer solution was added to the samples at an equal weight to volume basis (i.e., 19.3 gm sample + 19.3 ml of buffer). This was then homogenized to a thick, foamy slurry in a 50 ml test tube. Since this sample appeared to be very thick, the samples were centrifuged (10,400 x g, 30 minutes) which resulted in three phases (fat, liquid, solid). This system, therefore, could not be used to prepare the samples for ELISA assay since it would be impossible to know which fraction contained the antigen. Therefore, Dr. Scott studied numerous solvent-based protein separation methods for separating the fat from the proteins without damaging the antigen. He finally determined that using TriReagent containing Tween 20 was the best approach to emulsify the fat/lipid to solubilize the protein. During this stage, it was determined that the relatively low moisture content of the samples affected the sample extraction. Reconstitution in sample buffer was necessary.

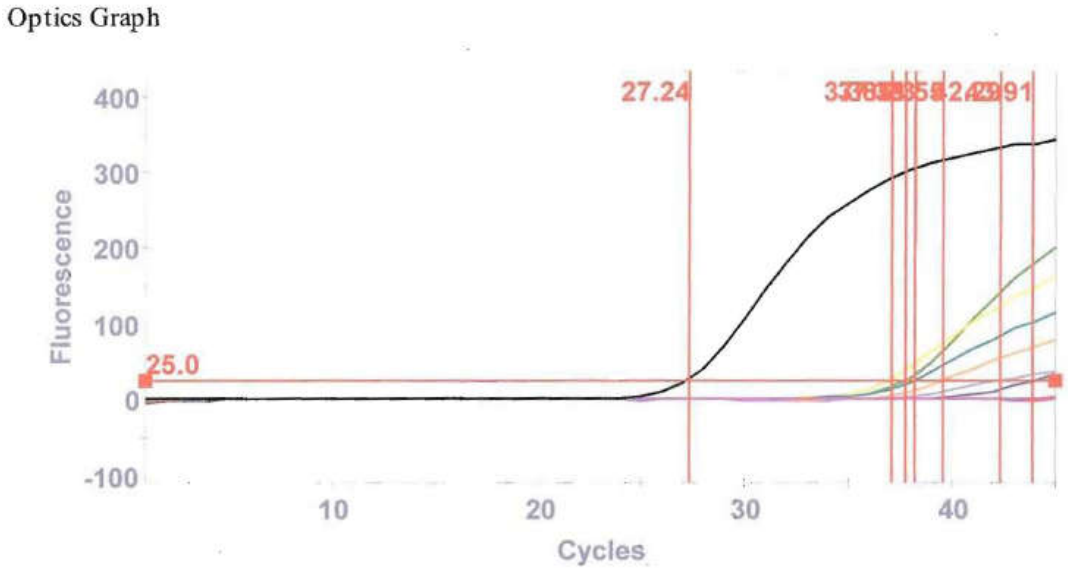
At this stage, it was determined that the reagents from the National Vet Lab were ineffective in samples involving extracted chicken tissues. It was determined that the National lab antibody is a chicken anti-AI and any second antibody used to detect this antibody will also detect other antibodies and immunoglobulins from the tissue extracts.

At this point, the researchers turned their attention to the polymerase chain reaction (PCR) assay for detecting the Type A influenza. Dr. Leaphart developed the following protocol:

1. Place approx. 3mL of rendered material in a 15mL Falcon tube.
2. Add 3mL of sterile Brain Heart Infusion broth and vortex to homogenize.

3. Spike sample with 50, 100, 250, 500, or 1000uL of influenza antigen prep and mix thoroughly by vortexing.
4. Extract RNA from (a) homogenized samples, approx. 500uL and (b) the oil layer from spun material, also 500uL.
5. Amplify and detect.

Dr. Leaphart's initial results are as follows:



Site Legend

Site ID	Sample ID	FAM Ct	Protocol
C1	50	43.91	AI-Matrix-rRT-PCR
C2	100	42.29	AI-Matrix-rRT-PCR
C3	250	39.55	AI-Matrix-rRT-PCR
C4	500	37.71	AI-Matrix-rRT-PCR
C5	1000	37.05	AI-Matrix-rRT-PCR
C6	50 oil	0.00	AI-Matrix-rRT-PCR
C7	100 oil	0.00	AI-Matrix-rRT-PCR
C8	250 oil	0.00	AI-Matrix-rRT-PCR
C9	500 oil	0.00	AI-Matrix-rRT-PCR
C10	1000 oil	38.23	AI-Matrix-rRT-PCR
C11	Neg	0.00	AI-Matrix-rRT-PCR
C12	Pos	27.24	AI-Matrix-rRT-PCR

Data Type	Line Type
Primary Curve	———

Channel	Symbol
FAM	None

After initial trials, Dr. Leaphart performed additional experiments to get the ratio of antigen to material correct for a strong enough signal. The breakdown was as follows:

Start with 500 uL rendered material plus 500 uL of brain heart infusion broth. Add the following amounts of antigen prep to produce the resulting signal:

0 uL; Ct = 0

50 uL; Ct = 38.68

100 uL; Ct = 37.03

250 uL; Ct = 35.88

500 uL; Ct = 34.00

In his initial studies, Dr. Leaphart concluded that the addition of 250 uL of antigen prep produced the strongest signal without too much dilution of the rendered material. He concluded that the cooked material would have to be diluted with the brain heart infusion broth in order to successfully extract RNA. His assay method would use 1 mL of rendered material spiked with 500 uL of antigen prep (i.e. the same ratio as the 250 uL sample listed above).

For controls, Dr. Leaphart ran reagent (transcribed RNA) and nucleic acid extraction controls (pure antigen prep) to account for any disparities in detection. Positive and negative experimental controls were included as well for each rendering sample. The positive control was spiked with the same amount of antigen as the experimental samples, but not exposed to heat. The negative control was spiked with brain heart infusion broth and run in tandem with the experimental samples.

Samples of cooked poultry rendering materials were collected from three rendering plants in three southeastern states (South Carolina, Georgia, and North Carolina). Rendering samples were placed in a commercial food processor and blended to smooth. Samples were analyzed for chemical composition. Results of chemical analyses for Plant A, Plant B and Plant C are indicated in Tables 2, 3, and 4, respectively.

Table 2. Chemical analyses of rendered poultry sample from Plant A.

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
CRUDE PROTEIN	21.6 %	22.0 %

* VALUES CALCULATED FROM CURRENT RESEARCH FORMULAS

MINERAL ANALYSIS

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
P	0.60 %	0.61 %
K	0.20 %	0.20 %
Ca	0.92 %	0.94 %
Mg	0.04 %	0.04 %
S	0.18 %	0.18 %
Zn	30 ppm	31 ppm
Cu	10 ppm	10 ppm
Mn	3 ppm	3 ppm
Fe	57 ppm	58 ppm
Na	ppm	ppm
Ca/P	1.53	1.53

OTHER ANALYSES

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
FAT	65.0 %	66.2 %
NO3-N	ppm	ppm
ASH	2.1 %	2.1 %
MOISTURE		1.8 %
DRY MATTER		98.2 %

*** This lab is a participant in the North American Proficiency Testing Program, Association of American Feed Control Officials, and the National Forage Testing Association sample exchange programs.

Table 3. Chemical analyses of rendered poultry sample from Plant B.

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
CRUDE PROTEIN	31.0 %	31.5 %

* VALUES CALCULATED FROM CURRENT RESEARCH FORMULAS

MINERAL ANALYSIS

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
P	0.88 %	0.89 %
K	0.40 %	0.41 %
Ca	1.22 %	1.24 %
Mg	0.06 %	0.06 %
S	0.35 %	0.36 %
Zn	41 ppm	42 ppm
Cu	11 ppm	11 ppm
Mn	43 ppm	43 ppm
Fe	123 ppm	125 ppm
Na	ppm	ppm
Ca/P	1.39	1.39

OTHER ANALYSES

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
FAT	47.8 %	48.5%
NO3-N	ppm	ppm
ASH	6.8 %	6.9 %

MOISTURE_____ 1.5 %
DRY MATTER_____98.5 %

*** This lab is a participant in the North American Proficiency Testing Program, Association of American Feed Control Officials, and the National Forage Testing Association sample exchange programs.

Table 4. Chemical analyses of rendered poultry sample from Plant C.

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
CRUDE PROTEIN	26.5 %	26.9 %

* VALUES CALCULATED FROM CURRENT RESEARCH FORMULAS

MINERAL ANALYSIS

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
P	0.43 %	0.44 %
K	0.36 %	0.37 %
Ca	0.29 %	0.29 %
Mg	0.04 %	0.04 %
S	0.32 %	0.32 %
Zn	35 ppm	36 ppm
Cu	3 ppm	3 ppm
Mn	2 ppm	2 ppm
Fe	60 ppm	60 ppm
Na	ppm	ppm
Ca/P	0.67	0.67

OTHER ANALYSES

	DETERMINED AS-SAMPLED	100PERCENT DRY-MATTER
FAT	60.5 %	61.4%
NO3-N	ppm	ppm
ASH	4.9 %	5.0 %
MOISTURE		1.4 %
DRY MATTER		98.6%

*** This lab is a participant in the North American Proficiency Testing Program, Association of American Feed Control Officials, and the National Forage Testing Association sample exchange programs.

After the PCR assay was confirmed, samples were heat treated. Dr. Tom Scott, Mr. Laine Chambers and Dr. Annel Greene traveled to the Clemson University Livestock & Poultry Health/Veterinary Diagnostic Center in Columbia, SC to Dr. Adam Leaphart's laboratory. There the team conducted thermal treatments on spiked samples of rendering materials. Samples of rendering materials (above) were prepared in 2 ml polypropylene microcentrifuge tubes. Tubes were placed in a heating block on a digital heating unit. The experimental design selected for the study is shown in Table 1. Samples were heated to 50°C, 60°C, 70°C, 80°C, 90°C, 100°C, 110°C and 120°C for 0, 10, 20, 30, and 60 seconds. Temperature was confirmed prior to starting the timer.

Table 1. Experimental Design for Thermal Trials

			Plant A		Plant B		Plant C	
Temperature	Time		Dosed	Non-Dosed	Dosed	Non-Dosed	Dosed	Non-Dosed
50C (122F)	0 sec	Rep 1	A-21	A-91	B-21	B-91	C-21	C-91
		Rep 2	A-22	A-92	B-22	B-92	C-22	C-92
	10 sec	Rep 1	A-23	A-93	B-23	B-93	C-23	C-93
		Rep 2	A-24	A-94	B-24	B-94	C-24	C-94
	20 sec	Rep 1	A-25	A-95	B-25	B-95	C-25	C-95
		Rep 2	A-26	A-96	B-26	B-96	C-26	C-96
	30 sec	Rep 1	A-27	A-97	B-27	B-97	C-27	C-97
		Rep 2	A-28	A-98	B-28	B-98	C-28	C-98
	60 sec	Rep 1	A-29	A-99	B-29	B-99	C-29	C-99
		Rep 2	A-30	A-100	B-30	B-100	C-30	C-100
60C (140F)	0 sec	Rep 1	A-51	A-121	B-51	B-121	C-51	C-121
		Rep 2	A-52	A-122	B-52	B-122	C-52	C-122
	10 sec	Rep 1	A-53	A-123	B-53	B-123	C-53	C-123
		Rep 2	A-54	A-124	B-54	B-124	C-54	C-124
	20 sec	Rep 1	A-55	A-125	B-55	B-125	C-55	C-125
		Rep 2	A-56	A-126	B-56	B-126	C-56	C-126
	30 sec	Rep 1	A-57	A-127	B-57	B-127	C-57	C-127
		Rep 2	A-58	A-128	B-58	B-128	C-58	C-128
	60 sec	Rep 1	A-59	A-129	B-59	B-129	C-59	C-129
		Rep 2	A-60	A-130	B-60	B-130	C-60	C-130
70C (158F)	0 sec	Rep 1	A-61	A-131	B-61	B-131	C-61	C-131
		Rep 2	A-62	A-132	B-62	B-132	C-62	C-132
	10 sec	Rep 1	A-63	A-133	B-63	B-133	C-63	C-133
		Rep 2	A-64	A-134	B-64	B-134	C-64	C-134
	20 sec	Rep 1	A-65	A-135	B-65	B-135	C-65	C-135
		Rep 2	A-66	A-136	B-66	B-136	C-66	C-136
	30 sec	Rep 1	A-67	A-137	B-67	B-137	C-67	C-137
		Rep 2	A-68	A-138	B-68	B-138	C-68	C-138
	60 sec	Rep 1	A-69	A-139	B-69	B-139	C-69	C-139
		Rep 2	A-70	A-140	B-70	B-140	C-70	C-140

80C (176F)	0 sec	Rep 1	A-31	A-101	B-31	B-101	C-31	C-101
		Rep 2	A-32	A-102	B-32	B-102	C-32	C-102
	10 sec	Rep 1	A-33	A-103	B-33	B-103	C-33	C-103
		Rep 2	A-34	A-104	B-34	B-104	C-34	C-104
	20 sec	Rep 1	A-35	A-105	B-35	B-105	C-35	C-105
		Rep 2	A-36	A-106	B-36	B-106	C-36	C-106
	30 sec	Rep 1	A-37	A-107	B-37	B-107	C-37	C-107
		Rep 2	A-38	A-108	B-38	B-108	C-38	C-108
	60 sec	Rep 1	A-39	A-109	B-39	B-109	C-39	C-109
		Rep 2	A-40	A-110	B-40	B-110	C-40	C-110
90C (194F)	0 sec	Rep 1	A-1	A-71	B-1	B-71	C-1	C-71
		Rep 2	A-2	A-72	B-2	B-72	C-2	C-72
	10 sec	Rep 1	A-3	A-73	B-3	B-73	C-3	C-73
		Rep 2	A-4	A-74	B-4	B-74	C-4	C-74
	20 sec	Rep 1	A-5	A-75	B-5	B-75	C-5	C-75
		Rep 2	A-6	A-76	B-6	B-76	C-6	C-76
	30 sec	Rep 1	A-7	A-77	B-7	B-77	C-7	C-77
		Rep 2	A-8	A-78	B-8	B-78	C-8	C-78
	60 sec	Rep 1	A-9	A-79	B-9	B-79	C-9	C-79
		Rep 2	A-10	A-80	B-10	B-80	C-10	C-80
100C(212F)	0 sec	Rep 1	A-11	A-81	B-11	B-81	C-11	C-81
		Rep 2	A-12	A-82	B-12	B-82	C-12	C-82
	10 sec	Rep 1	A-13	A-83	B-13	B-83	C-13	C-83
		Rep 2	A-14	A-84	B-14	B-84	C-14	C-84
	20 sec	Rep 1	A-15	A-85	B-15	B-85	C-15	C-85
		Rep 2	A-16	A-86	B-16	B-86	C-16	C-86
	30 sec	Rep 1	A-17	A-87	B-17	B-87	C-17	C-87
		Rep 2	A-18	A-88	B-18	B-88	C-18	C-88
	60 sec	Rep 1	A-19	A-89	B-19	B-89	C-19	C-89
		Rep 2	A-20	A-90	B-20	B-90	C-20	C-90

Preliminary results are as follows:

Plant A

50 C
A-21 :Ct=0
22 :38.21
23 :36.00
24 :34.30
25 :34.95
26 :37.21
27 :36.07
28 :40.18
29 :35.77
30 :36.89

60 C
A-51 :Ct=54.62
52 :37.13
53 :35.96
54 :37.76
55 :36.64
56 :35.46
57 :38.62
58 :41.94
59 :34.91
60 :34.99

70 C
A-61 :Ct=41.51
62 :40.64
63 :52.57
64 :36.56
65 :39.69
66 :37.25
67 :37.57
68 :38.12
69 :38.20
70 :38.32

80 C
A-31 :Ct=36.33
32 :40.96
33 :37.39
34 :39.96
35 :41.93
36 :38.17
37 :37.40
38 :47.37
39 :39.59
40 :39.11

Plant B

80 C
B-31 :Ct=38.99
32 :38.37
33 :37.30
34 :36.18
35 :36.49
36 :37.31

37 :35.29
38 :35.38
39 :36.30
40 :35.75

Plant C

80 C
C-31 :Ct=35.58
32 :37.91
33 :36.02
34 :35.19
35 :35.59
36 :33.69
37 :34.61
38 :35.38
39 :36.57
40 :34.63

Analyses of 50°C, 60°C, 70°C, and 80°C heat treated samples indicated no destruction of the Type A influenza. Difficulties in obtaining temperatures above 100ºC occurred in the experiment due to boiling out of the short polypropylene tubes. In addition, the fat caused premature melting of the polypropylene below the rated melt temp of the tubes. Therefore, the researchers searched for stainless steel test tubes only to discover that they were no longer manufactured. Therefore, they had stainless steel test tubes manufactured by a local fabrication company. The experiment was repeated as above but the temperatures used were 110°C (230°F), 120°C (248°F), 130°C (266°F), and 140°C (284°F) for 0, 15, 30, 60 and 120 seconds.

Results are shown below:

			Plant A				Plant B				Plant C			
Temp	Time	Rep	Dosed	Ct	Non-Dosed	Ct	Dosed	Ct	Non-Dosed	Ct	Dosed	Ct	Non-Dosed	Ct
110C	0 sec	Rep 1	A-1	39.91	A-41	0	B-81	36.66	B-121	0	C-161	36.21	C-201	0
(230F)		Rep 2	A-2	40.39	A-42	0	B-82	38.14	B-122	0	C-162	34.97	C-202	0
	15 sec	Rep 1	A-3	47.15	A-43	0	B-83	50.73	B-123	0	C-163	0	C-203	0
		Rep 2	A-4	0	A-44	0	B-84	39.7	B-124	0	C-164	0	C-204	0
	30 sec	Rep 1	A-5	0	A-45	0	B-85	0	B-125	0	C-165	45.32	C-205	0
		Rep 2	A-6	0	A-46	0	B-86	0	B-126	0	C-166	41.38	C-206	0
	60 sec	Rep 1	A-7	0	A-47	0	B-87	0	B-127	0	C-167	40.62	C-207	0
		Rep 2	A-8	0	A-48	0	B-88	0	B-128	0	C-168	0	C-208	0
	120 sec	Rep 1	A-9	0	A-49	0	B-89	54.62	B-129	0	C-169	0	C-209	0
		Rep 2	A-10	47.56	A-50	0	B-90	0	B-130	0	C-170	0	C-210	0
120C	0 sec	Rep 1	A-11	34.94	A-51	0	B-91	36.31	B-131	0	C-171	35.27	C-211	0
(248F)		Rep 2	A-12	34.75	A-52	0	B-92	38.31	B-132	0	C-172	34.94	C-212	0
	15 sec	Rep 1	A-13	41.55	A-53	0	B-93	39.7	B-133	0	C-173	0	C-213	0
		Rep 2	A-14	0	A-54	0	B-94	0	B-134	0	C-174	49.23	C-214	0
	30 sec	Rep 1	A-15	0	A-55	0	B-95	0	B-135	0	C-175	0	C-215	0
		Rep 2	A-16	0	A-56	0	B-96	0	B-136	0	C-176	47.33	C-216	0
	60 sec	Rep 1	A-17	0	A-57	0	B-97	0	B-137	0	C-177	0	C-217	0
		Rep 2	A-18	0	A-58	0	B-98	0	B-138	0	C-178	0	C-218	0
	120 sec	Rep 1	A-19	0	A-59	0	B-99	0	B-139	0	C-179	0	C-219	0
		Rep 2	A-20	0	A-60	0	B-100	54.47	B-140	0	C-180	0	C-220	0
130C	0 sec	Rep 1	A-21	36.44	A-61	0	B-101	36.36	B-141	0	C-181	36.55	C-221	0
(266F)		Rep 2	A-22	37.52	A-62	0	B-102	37	B-142	0	C-182	35.44	C-222	0
	15 sec	Rep 1	A-23	0	A-63	0	B-103	0	B-143	0	C-183	0	C-223	0
		Rep 2	A-24	0	A-64	0	B-104	0	B-144	0	C-184	0	C-224	0
	30 sec	Rep 1	A-25	0	A-65	0	B-105	0	B-145	0	C-185	0	C-225	0
		Rep 2	A-26	0	A-66	0	B-106	0	B-146	0	C-186	0	C-226	0
	60 sec	Rep 1	A-27	0	A-67	0	B-107	0	B-147	0	C-187	52.35	C-227	0
		Rep 2	A-28	0	A-68	0	B-108	0	B-148	0	C-188	42.48	C-228	0
	120 sec	Rep 1	A-29	0	A-69	0	B-109	0	B-149	0	C-189	0	C-229	0
		Rep 2	A-30	0	A-70	0	B-110	0	B-150	0	C-190	0	C-230	0
140C	0 sec	Rep 1	A-31	37.43	A-71	0	B-111	39.94	B-151	0	C-191	34.42	C-231	0
(284F)		Rep 2	A-32	41.78	A-72	0	B-112	37.64	B-152	0	C-192	33.07	C-232	0
	15 sec	Rep 1	A-33	0	A-73	0	B-113	0	B-153	0	C-193	0	C-233	0
		Rep 2	A-34	0	A-74	0	B-114	0	B-154	0	C-194	0	C-234	0
	30 sec	Rep 1	A-35	0	A-75	0	B-115	0	B-155	0	C-195	0	C-235	0
		Rep 2	A-36	0	A-76	0	B-116	0	B-156	0	C-196	0	C-236	0
	60 sec	Rep 1	A-37	0	A-77	0	B-117	0	B-157	0	C-197	0	C-237	0
		Rep 2	A-38	0	A-78	0	B-118	0	B-158	0	C-198	0	C-238	0
	120 sec	Rep 1	A-39	0	A-79	0	B-119	0	B-159	0	C-199	0	C-239	0
		Rep 2	A-40	0	A-80	0	B-120	48.07	B-160	0	C-200	0	C-240	0

Positve Control (transcribed RNA) Ct = 26.63
Negative Control Ct = 0
Extraction Control (antigen prep) Ct = 27.38

Positve Control (transcribed RNA) Ct = 25.74
Negative Control Ct = 0
Extraction Control (antigen prep) Ct = 29.66

Positve Control (transcribed RNA) Ct = 26.82
Negative Control Ct = 0
Extraction Control (antigen prep) Ct = 30.86

Positve Control (transcribed RNA) Ct = 26.26
Negative Control Ct = 0
Extraction Control (antigen prep) Ct = 28.89

This project is designed to determine the least thermal treatment that will destroy avian influenza in rendered products. Due to the nature of the test procedure, the polymerase chain reaction assay used in this experiment is designed to determine if any Type A influenza is destroyed in the sample. This assay indicates a total melting of the RNA and not simply killing of the virus particle. Research conducted by Swain in Georgia indicated that avian influenza was killed at 70°C within a few seconds.

In our results, the mean Ct value (i.e., the reaction cycle at which the sample exceeds the threshold value and produces detectable CDNA) and associated standard errors for different combinations of times and temperatures. The means are based on three different plants with two reps each.

			Time			
Temperature	**0**	**15**	**30**	**60**	**120**	**Overall**
110°C	2.35(0.5)^{b***}	0.05(0.5)^{c}	0(0.5)^{c}	0(0.5)^{c}	0(0.5)^{c}	**0.48(0.25)^{a**}**
120°C	4.25(0.5)^{a}	0.05(0.5)^{c}	0(0.5)^{c}	0(0.5)^{c}	0(0.5)^{c}	**0.86(0.25)^{a}**
130°C	3.45(0.5)^{ab}	0(0.5)^{c}	0(0.5)^{c}	0(0.5)^{c}	0(0.5)^{c}	**0.69(0.25)^{a}**
140°C	2.92(0.5)^{ab}	0(0.5)^{c}	0(0.5)^{c}	0(0.5)^{c}	0(0.5)^{c}	**0.58(0.25)^{a}**
Overall	**3.24(0.27)^{a*}**	**0.02(0.27)^{b}**	**0(0.27)^{b}**	**0(0.27)^{b}**	**0 (0.27)^{b}**	

* overall time means with similar letters are not significantly different based on ANOVA followed by Fisher's Least Significance Test with α set at 0.05.

** overall temperature means with similar letters are not significantly different based on ANOVA followed by Fisher's Least Significance Test with α set at 0.05.

*** means for the time and temperature combinations with similar letters are not significantly different based on ANOVA followed by Fisher's Least Significance Test with α set at 0.05.

Results indicated that Type A influenza RNA was destroyed at rendering temperatures.

Impacts and Significance:

Validation of thermal destruction of avian influenza in rendered animal products is of critical importance in case of an outbreak of the disease.