ENSILING POULTRY CARCASSES FOR BIOSECURE PRESERVATION AND VIRUS DESTRUCTION

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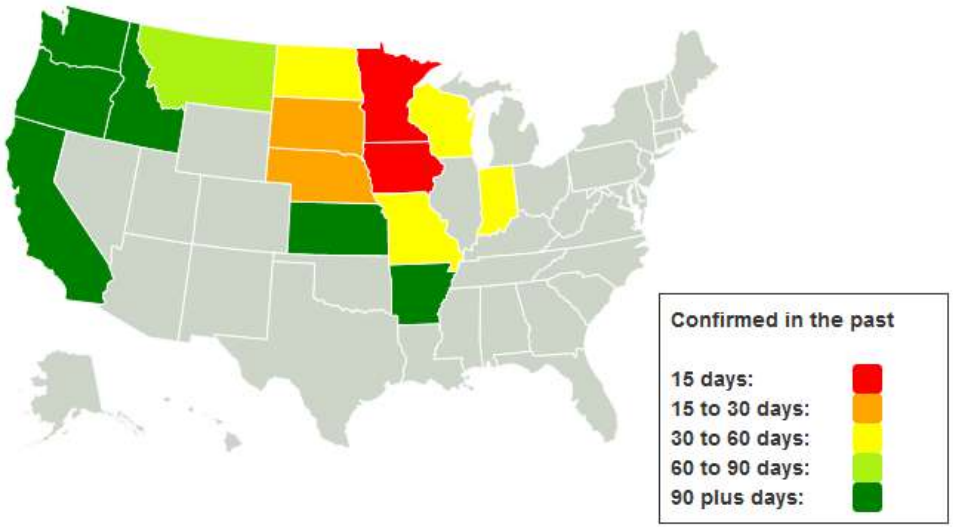
Outline

- Objective: To evaluate the ensiling of Leghorn hen carcasses as a means of virus destruction and biosecure carcass preservation
- Introduction
- Materials & Methods
- Results
- Summary

Findings as of July 15 2015

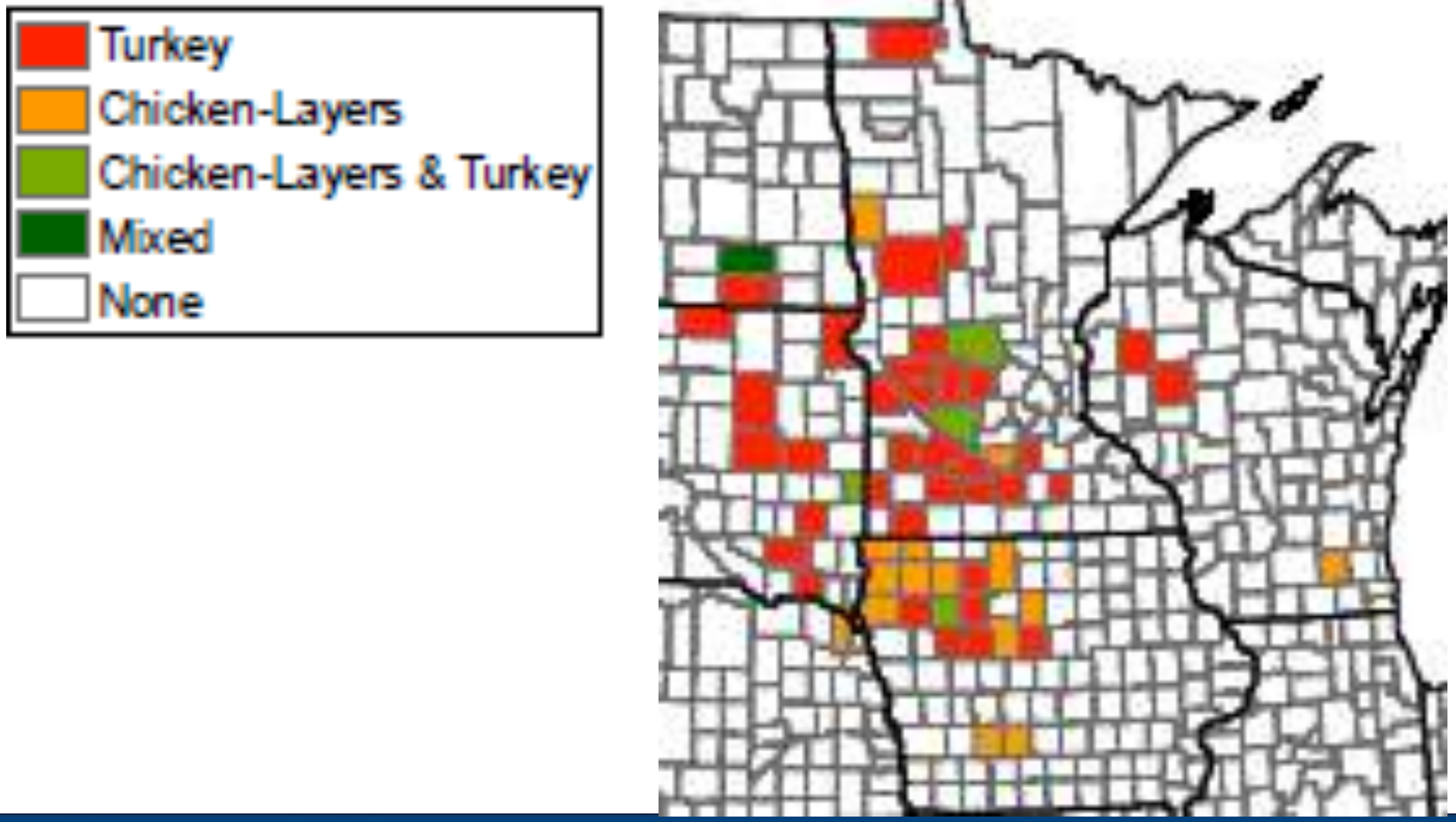
ALL Findings

Update on Avian Influenza Findings
Poultry Findings Confirmed by USDA's National Veterinary Services Laboratories



223 Detections Reported	**48,091,293** Birds Affected
12/19/14 First Detection Reported	**6/17/15** Last Detection Reported

Commercial Poultry



PA HPAI Task Force
Disposal Sub Committee

- Compost
- Render
- Incinerate
- Burial
- Landfill
- *Ensile*

- USDA “storage necessary” when euthanasia (24hr) exceeds disposal
- Storage:
  - Adequate stabilization, preservation
  - Not a fire, health or safety issue
  - No spillage, in closed leak proof containers
  - Adequate unit size, numbers & maintain clean conditions
  - Safeguards to protect water, soil & air from leachate, pathogens and gases

(FAD PReP-USDA SOP’s: 14. Disposal, 2014)

Previous Work

- Dobbins, D.W. 1988. Lactobacillus fermentation: A method of disposal utilization of carcasses or toxic chemicals. Pp. 76-80 Proceedings of the Natl. Poult Waste Mngt. Symp.
- Patterson, P.H., N. Acar & W.C. Coleman. 1994. Feeding value of poultry by-products extruded with cassava, barley, and wheat middlings for broiler chicks: The effect of ensiling poultry by-products as a preservation method prior to extrusion. PS 73:1107-1115.
- Deshmukh, A.C. and P.H. Patterson. 1997a&b. Preservation of hatchery waste by lactic acid fermentation. 1. Lab-scale & 2. Large-scale and feeding trial. PS 76:1212-1226.

Previous Work

- Kim W.K. & P.H. Patterson. 2000. Recycling dead hens by enzyme or sodium hydroxide pretreatment and fermentation. PS 79:879-885.

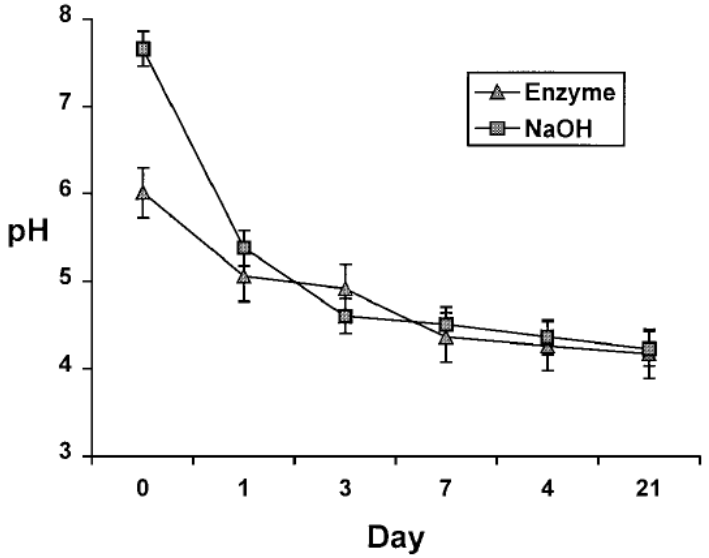


TABLE 2. Microbial analysis of enzyme- and NaOH-treated dead hens before and after a 21-d fermentation

Treatment	*Escherichia coli*	*Staphylococcus aureus*	Streptococcus	Lactobacillus
	(log cfu/g)			
Control	4.16[b]	4.04[b]	0.00[b]	2.55[b]
Enzyme treatment before fermentation	5.37[a]	5.13[a]	5.78[a]	4.81[ab]
NaOH treatment before fermentation	4.50[b]	4.17[b]	5.01[a]	2.68[b]
Enzyme treatment after fermentation	ND[1]	ND	4.63[a]	5.55[a]
NaOH treatment after fermentation	ND	ND	4.83[a]	6.23[a]
Pooled SEM	0.13	0.33	0.30	0.65

[a–b]Means within a column with different superscripts differ significantly ($\pm < 0.05$). n = 3 per mean.

[1]ND = not detectable.

Materials and Methods

- 65-wk old Leghorn fowl (5,460) were euthanized with CO_2 gas
- Carcasses ensiled in an “Ag Bag” 2.4x6.1m long
- 2 carbohydrate treatments: 14% hen feed, and mixture of 9% feed + 5% sugar were mixed and ensiled with the carcasses (2880/treat)
- Silage inoculant added to both treatments: 1.0E6 cfu/g silage L. *plantarum & P. acidilactici*

Penn State **Extension**

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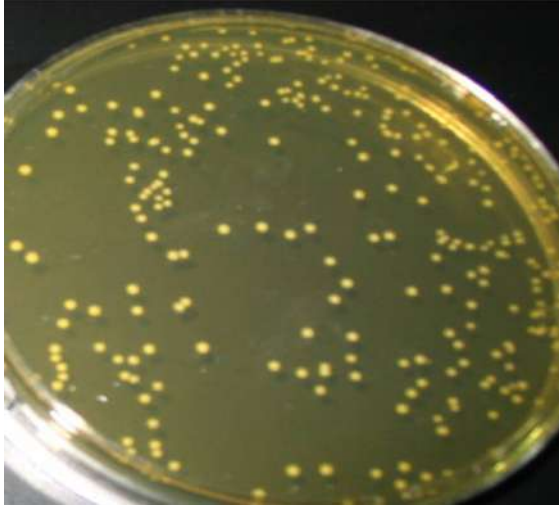
Materials and Methods

- 18 carcasses inoculated with AviPro SOHOL, live Newcastle-Bronchitis virus reconstituted in d water & suspended in BHI broth (40ml).
- 1ml was injected into the trachea, and 1ml in the cloaca (250 doses per ml)
- 3 bundles of 3 carcasses per poly-net bag placed in each treatment
- At 3, 7, 14 and 29d treatments were probed for pH, microbial samples & 29d virus isolation

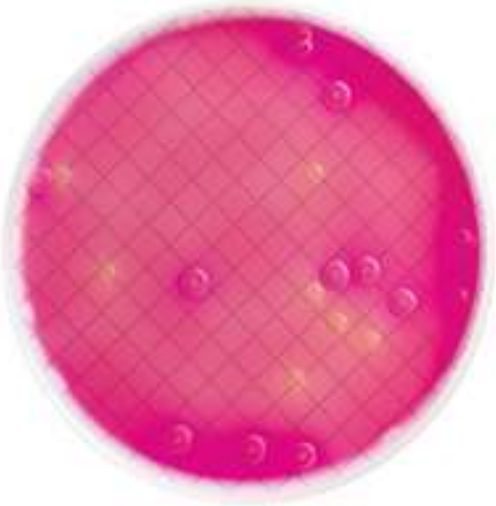
Penn State **Extension**

Bacterial Enumeration



- **Lactic acid bacteria counts**
  - de Man-Rogosa-Sharpe agar
  - Serial dilutions from 10^{-2} to 10^{-4}
  - Plated of 100 μl of each dilution
  - Incubate at 37°C, 48 hrs anaerobic conditions
  - CFU/ml = total colony count x 10 x dil factor

Bacterial Enumeration



- **Coliform counts**
  - MacConkey agar
  - Direct plate of 100 μl of sample
  - Incubate at 37°C for 18 hrs
  - Expressed as colony forming units/ml
  - CFU/ml = total colony count x 10

Chick Embryo ND Virus Isolation

- "Gold Std" for live virus

Tracheal/Cloacal Swabs

↓

VI in ECE (9-11 day)
@37C for 72-96 hrs

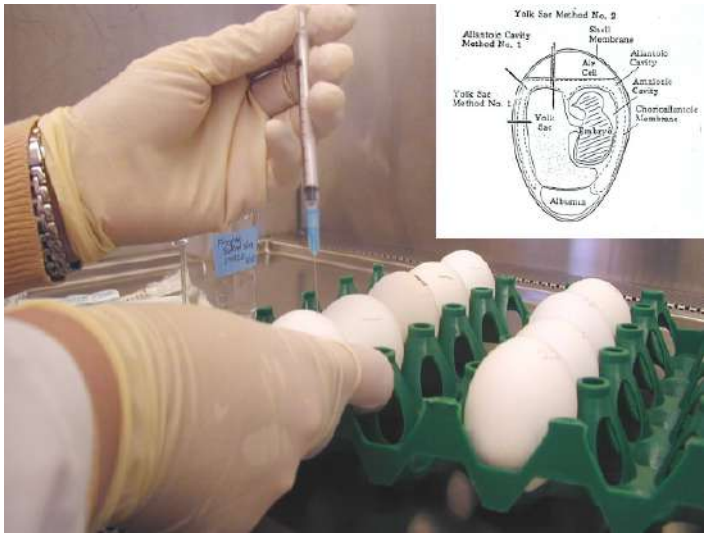
↓

Allantoic Fluid for HA test

↓

Virus Inactivated

Virus Live



Results: pH

Treatments	Day 3	Day 7	Day 14	Day 29
Feed	5.38	6.25	6.25	5.88
Feed+Sugar	5.25	4.63	5.50	3.88
SEM	0.18	0.37	0.10	0.10
P-value	0.6480	0.0213	0.0020	<0.0001

Results: Microbial (cfu/ml)

Lactic acid bacteria

Treatments	Day 3	Day 7	Day 14	Day 29
Feed	1.052E8	9.200E6	1.625E6	1.975E7
Feed+Sugar	1.809E8	1.438E7	6.750E4	2.325E4
SEM	4.286E7	7.178E6	4.459E5	5.232E6
P-value	0.2582	0.6328	0.0485	0.1081

Coliforms

Treatments	Day 3	Day 7	Day 14	Day 29
Feed	130	10,175	0	0
Feed+Sugar	1,233,090	0	0	0
SEM	486,589	6,800	0	0
P-value	0.1234	0.3308	---	---

Results: ND Virus Isolation

Treatment	Trachea Pool	Cloaca Pool	Trachea Tubes	Cloaca Tubes
Feed	2 neg pool	2 neg pool	4 neg tube	9 neg tube
Feed+Sugar	2 neg pool	2 neg pool	8 neg tube	7 neg tube

* No positive samples

Summary

- Rapid drop, and sustained low pH (esp F+S)
- Bacteria:
  - Coliforms: eliminate by day 7 (Feed+Sugar) and day 14 and on in (Feed)
  - Lactic acid bacteria: Feed = 1.0E8-6, Feed+Sugar = 1.0E8-4
- Virus Isolation: No positive samples
  All trachea & cloaca pools & individual tubes Neg

Summary

- Maceration of carcasses may not require silage inoculant
- Meets the FAD-USDA guidelines for “storage”
- Mechanics & logistics of carcass handling +++
- Gas generation will require one-way valves

Summary

- Ensiling can scale-up for commercial application
- 100,000 hens, 10ft bag: 809 bds & 2630 lb/ft & 124 ft bag

Summary

- 1 mill hens, 14ft bag: 1588 bds & 5160 lb/ft & 630 ft bag