Project Title: Production and Processing Intervention Strategies for Poultry Associated Foodborne Pathogens

Project No: 6040-32000-069-00D

Location: Athens, GA

Scientists:

R. Jeff Buhr - Physiologist

Doug E. Cosby - Microbiologist

Nelson A. Cox - Microbiologist

Arthur Hinton - Microbiologist

Objective 1: Develop reliable and reproducible challenge models with *Salmonella* and *Campylobacter* for use in accurately developing, evaluating, and validating processes for reducing pathogen load using various chemical sanitizers.

1. Day-of-processing challenge model for the RRC pilot processing plant for *Salmonella* and *Campylobacter* utilized for poultry food safety research. (DEC)

Objective 2: Develop, evaluate, and validate current and novel chemicals, operational protocols, and sampling methodologies used during poultry production and processing of broilers for the reduction and/or control of foodborne pathogens.

2.1. Commercially available carcass sanitizer identified. (NAC)

2.2. Protocols and chemical applications during defeathering to lower *Salmonella* and *Campylobacter* on postchill carcasses. (DEC)

2.3. A novel poultry processing sanitizer formulated from medium-chained-fatty acids and organic acids. (AH)

Objective 3: Identify and evaluate risk factors in the production, management, transportation, or processing that impact bird/egg contamination with foodborne pathogens and develop intervention strategies to control pathogens in the absence of antibiotics.

3.1. Effective chemical sanitizer for disinfecting fertile hatching eggs. (RJB)

3.2. Commercial broiler antibiotic free feeding programs that enhance resistance to *Salmonella* and *Campylobacter* (RJB)

Objective 4: Determine extrinsic factors that impact the survival and attachment of microorganisms including evaluating media and growth factors. Develop and validate new improved technologies to isolate and propagate foodborne pathogens.

4.1. New bacteriological medium that will support growth of *Campylobacter* cultures incubated aerobically. (AH)

4.2. Effective *Salmonella* isolation method identified. (NAC)

Project No: 6040-32000-069-00D
Start Date: 02/10/2016

	2016 Final Report	2017	2018
Accomplishments	2	5	2
Technology Transfer	3	0	1
Manuscripts	13	15	15

Accomplishments: 2017

1) Novel selective, bacteriological medium formulated to support growth of *Campylobacter*.

2) Isolation and identification of amylase-producing, endospore-forming bacteria for use in defined probiotic cultures.

3) *Development of neutralizing Buffered Peptone Water (nBPW) to inactivate chemical sanitizers in *Salmonella* verification studies.

Accomplishments: 2017

4) Respiratory tracts of broilers are not significantly contaminated with *Salmonella*, aerobic, or Enterobacter bacteria following house preparation and catching.

5) Electrically stunning shackled broilers using a combination of pulsed DC (salt-water bath for 10 seconds) followed by the application of AC (contact plate for 5 seconds) confirmed 100% efficacy in induction of a non-recoverable stun-to death.

Development of Neutralizing Buffered Peptone Water for *Salmonella* Verification Testing in Commercial Poultry Processing Facilities

Mark Berrang, Jeff Buhr, Gary Gamble, Arthur Hinton - ARS
+
John J. Johnston - FSIS

Journal of Food Protection 79: 2016, 710-714.
doi:10.4315/0362-028X.JFP-15-461
Journal of Food Protection 80: 2017, 685–691.
doi:10.4315/0362-028X.JFP-16-412
Poultry Science 96: 2017, 241-245.
doi.org/10.3382/ps/pew275

BACKGROUND

- FSIS requested that ARS scientists at the U. S. National Poultry Research Center conduct research on the effect of sanitizer carryover on the recovery of *Salmonella*.

- Sanitizers
    - Acid mixtures
    - Acidified sodium chlorite (ASC)
    - Cetylpyridinium chloride (CPC)
    - 1,3-dibromo-5,5-dimethylhydantion (DBDMH)
    - Peroxyacetic acid (PAA).

- Scientists determined that sanitizer carry-over could reduce recovery of *Salmonella*.

nBPW

- ARS scientists formulated a new Buffered Peptone Water (nBPW) that is now used in *Salmonella* verification testing in commercial poultry processing the U.S.

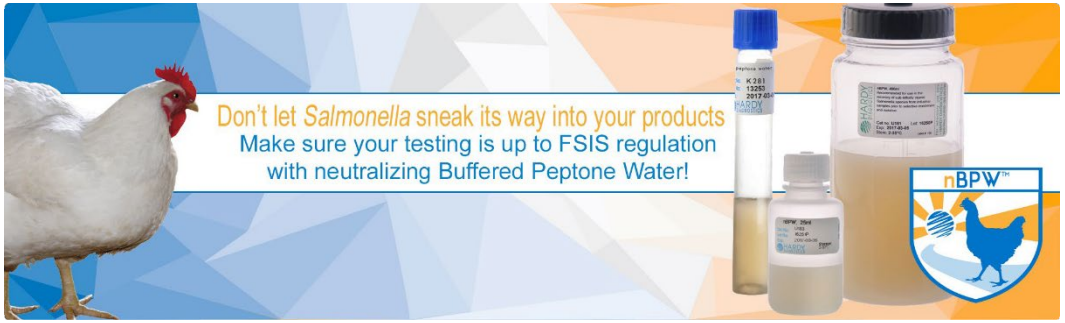
- Composition

  Buffered peptone-20.0 g
  Refined soy lecithin or equivalent-7 g
  Sodium thiosulfate-1 g
  Sodium bicarbonate12.5g
  Water-1 L

Technology Transfer

- FSIS issued Notice 41-16 on June 8, 2016 to inform commercial poultry processing facilities of adoption of nBPW.

- The product is now commercially manufactured and distributed.



Impact

- The nBPW will be used to conduct approximately 27,000 poultry *Salmonella* verifications tests per year nationwide.

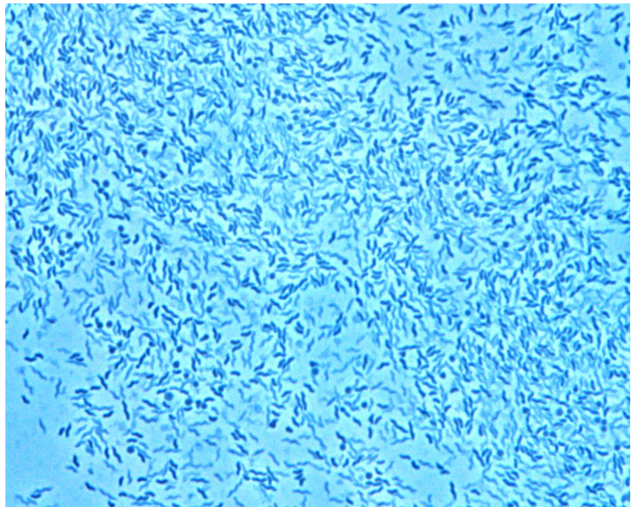
- Improved verification testing will increase the confidence of FSIS in the results of the Agency's *Salmonella* testing data.

Accomplishments: 2018

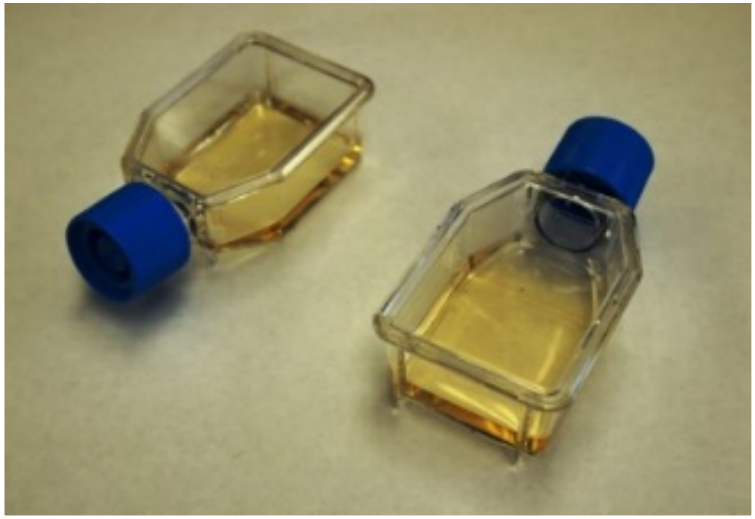
1) *Novel selective medium formulated to isolate *Campylobacter* from mixed cultures.

2) *The time of day that poultry house litter is sampled does not impact the predicted *Salmonella* or *Campylobacter* status of the flock.

Development of New, Selective Medium for Aerobic Recovery of *Campylobacter* from Mixed Bacterial Cultures



Campylobacter



Campy Media in Flasks

Basal Medium Composition

Beef extract- 50 g
Tryptose- 10 g
Soluble starch-10 g
Sodium lactate- 3.0 g
Agar- 0.5 g
Distilled Water- 900 ml

Selective Media

Basal medium supplemented with 1 of the following antibiotic mixtures currently used in selective *Campylobacter* media:

- **Cefex**
- **Bolton**
- **Skirrow**

Isolates

- ***Campylobacter* species**
  - ***C. coli***
  - ***C. fetus***
  - ***C. jejuni***
  - ***C. lari***

- **Other bacteria associated with poultry**
  - ***Escherichia coli***
  - ***Enterococcus faecalis***
  - ***Listeria monocytogenes***
  - ***Pseudomonas aeruginosa***
  - ***Salmonella* Kentucky**
  - ***Staphylococcus aureus***

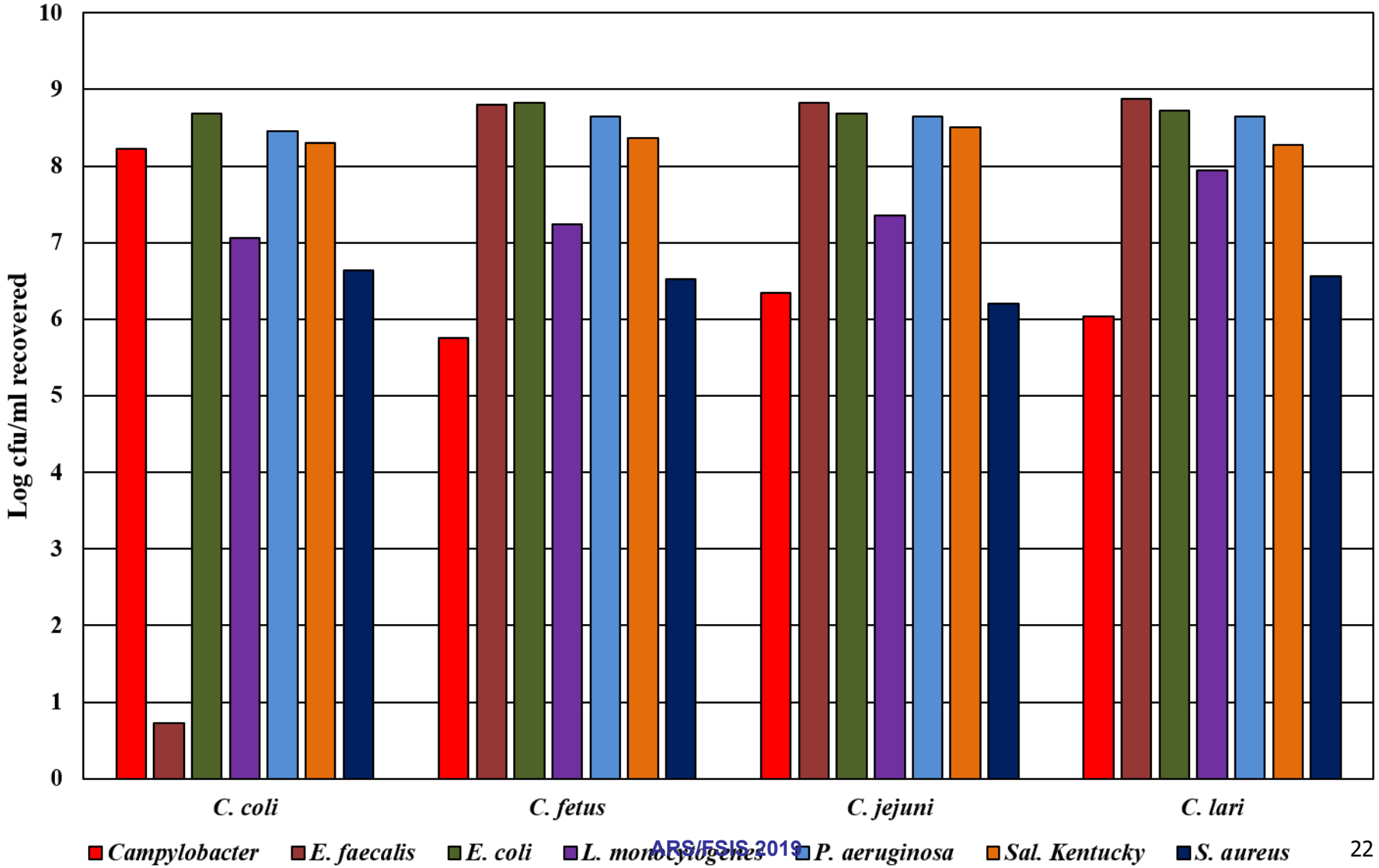
Growth of Mixed Cultures

- **Basal and selective media inoculated with mixed culture containing 10^3 cfu/ml one *Campylobacter* isolate with**
  - ***E. Coli***
  - ***E. Faecalis***
  - ***L. Monocytogenes***
  - ***P. Aeruginosa***
  - ***Salmonella* Kentucky**
  - ***S. aureus*.**

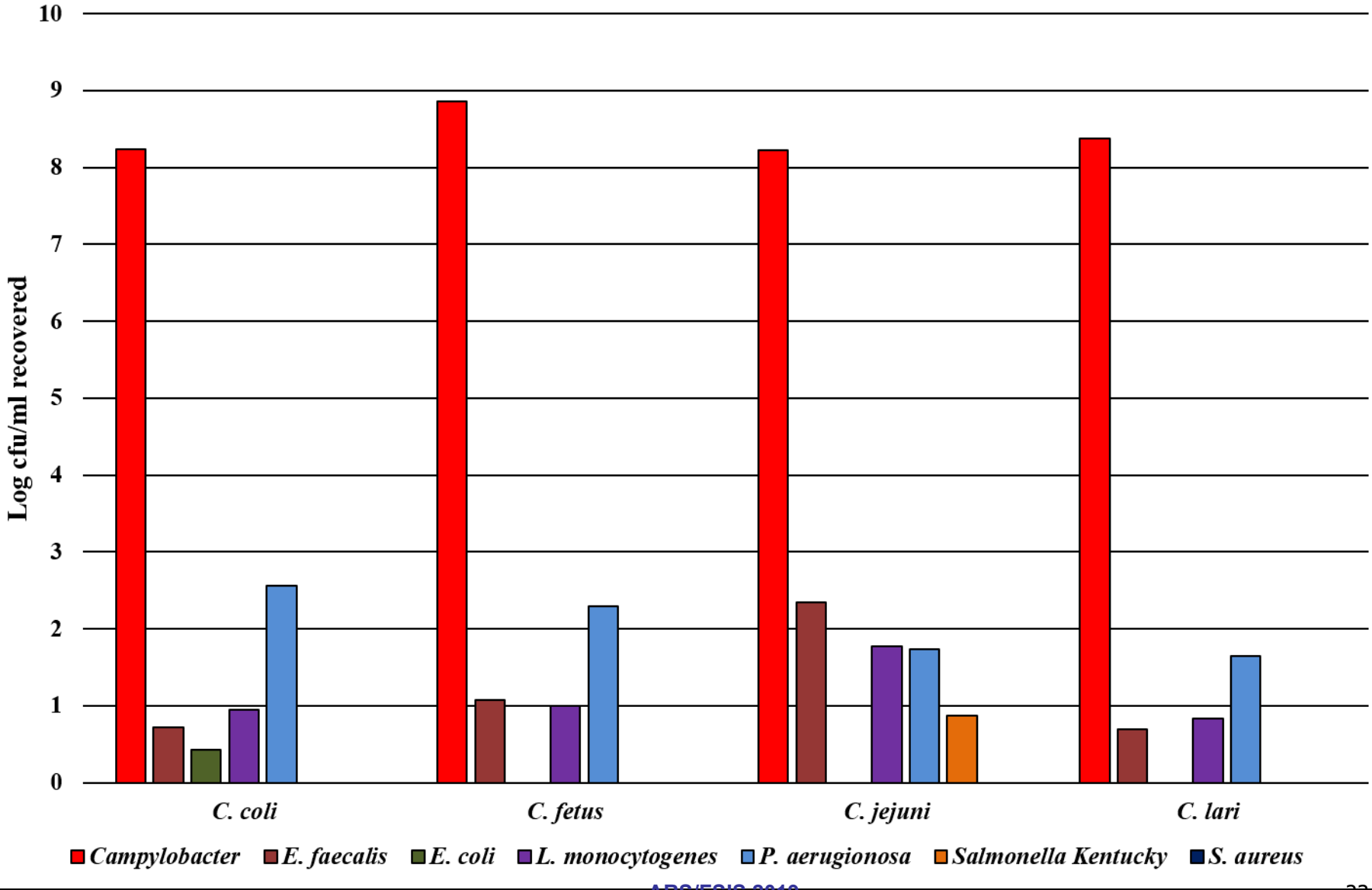
- **Cultures incubated aerobically at 37^{o}C for 48 h.**

- **Isolates enumerated on selective, differential agar media.**

Basal Medium



Selective Medium w/ Bolton Antibiotics



CONCLUSION

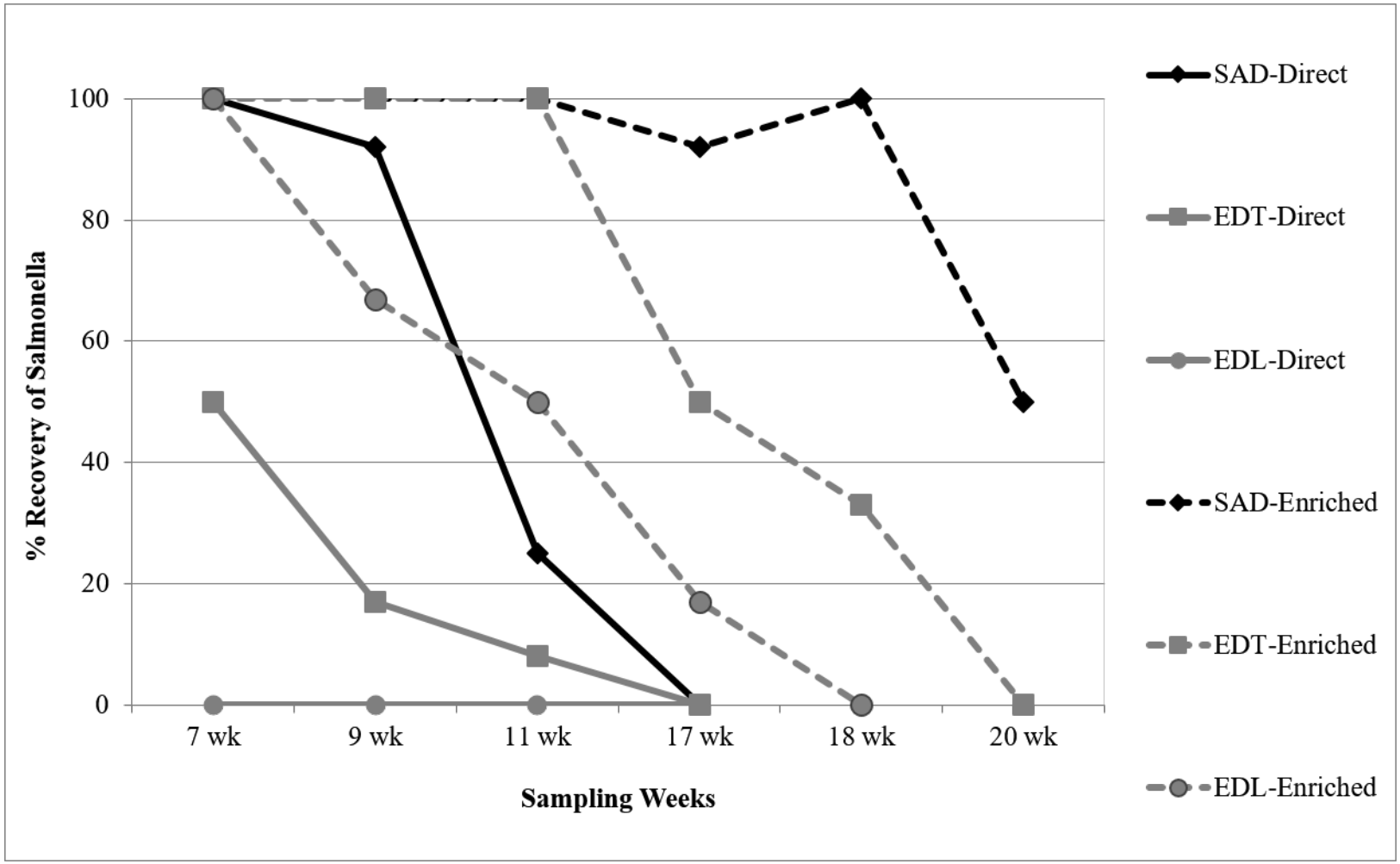
Basal medium supplemented with the Bolton antibiotic mixture is a selective media that can be incubated aerobically to isolate *Campylobacter* from samples containing other bacteria.

The time of day that poultry house litter is sampled does not impact the predicted *Salmonella* or *Campylobacter* status of the flock.

Question:
Observation of pen litter prior to the scheduled lights turning on (pullets were cooped for sampling 5 min early to minimize litter pecking the morning of sampling) revealed the presence of numerous fecal droppings that were rapidly trampled by the pullets with the onset of light.

Poultry Science 97: 2018, 2775–2784.
doi.org/10.3382/ps/pey150

The time of day that poultry house litter is sampled does not impact the predicted *Salmonella* or *Campylobacter* status of the flock.



Poultry Science 97: 2018, 2775–2784.
doi.org/10.3382/ps/pey150

CONCLUSION

Liter sampled at:

09:00	100% positive
15:00	100% positive
18:00	100% positive

Salmonella at 9, 11 and 17 wk

Campylobacter at 17 and 18 wk

For all feeding programs with enrichment:

Skip-a-Day in Feed Trough
Every-Day in Feed Trough
Every-Day on Litter Surface

Accomplishments: 2019

1) *High pressure washer to remove *Salmonella* from broiler carcasses.
2) *Survival of *Salmonella* and *Campylobacter* overnight 4ºC in cetylpyridinium chloride
3) *Evaluation of poultry water treatments during feed and water withdrawal on water usage and *Salmonella* prevalence in broilers
4) *Mobile Bird Euthanasia Apparatus

High pressure washer to remove Salmonella from broiler carcasses

Mean counts from pre- and post- wash breast swabs at three sites on the kill line for total aerobes, E. coli, and Enterobacteriaceae.

Rep	Site	Pre / Post Wash	Mean Log_{10} CFU/mL		
			Total Aerobic	E. coli	Enterobacteriaceae
1	Pre- Scald	Pre	7.42	4.81	4.98
1	Pre- Scald	Post	7.01	4.82	5.08
1	Post Scald	Pre	6.57^{A}	4.12^{A}	4.37^{A}
1	Post Scald	Post	5.17^{B}	3.12^{B}	3.42^{B}
1	Post Picker	Pre	4.53	1.92	3.02
1	Post Picker	Post	3.93	1.48	2.09
2	Pre- Scald	Pre	7.04	4.66	5.07
2	Pre- Scald	Post	6.75	3.72	4.48
2	Post Scald	Pre	5.7	3.03^{A}	3.22^{A}
2	Post Scald	Post	5.19	2.33^{B}	2.44^{B}
2	Post Picker	Pre	4.38	2.6	2.82
2	Post Picker	Post	3.94	1.98	2.26
3	Pre- Scald	Pre	8.51^{A}	5	6.04
3	Pre- Scald	Post	6.32^{B}	3.76	4.5
3	Post Scald	Pre	5.55	2.48	3.21
3	Post Scald	Post	5.35	2	2.92
3	Post Picker	Pre	4.19	2.18	2.74
3	Post Picker	Post	3.83	1.22	1.95

Overall means of the log_{10} converted CFU's.

	Pre / Post Wash	Pre-Scald*	Post-Scald	Post-Pick	Overall Mean
Total Aerobes	Pre-Wash	7.7^{A}	5.9^{C}	4.4	6.0
Total Aerobes	Post-Wash	6.7^{B}	5.0^{D}	3.9	5.2
E. coli	Pre-Wash	4.8	3.2	2.2	3.4
E. coli	Post-Wash	4.1	2.5	1.6	2.7
Enterobacteriaceae	Pre-Wash	5.4	3.6	2.8	4.0
Enterobacteriaceae	Post-Wash	4.6	2.9	2.1	3.2

$^{A-D}$ Values with different superscripts are significantly different.

* Log_{10} CFU/mL averaged across three replications.

CONCLUSION

Overall reductions ~ 0.8 log

Survival of *Salmonella* and *Campylobacter* overnight 4°C in cetylpyridinium chloride ppm

Survival of *Salmonella* and *Campylobacter* in buffered peptone water and CPC

Maximum concentration allowing growth of *Campylobacter* species.							
Isolate	Maximum concentration						
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Avg
C. coli - 5CC	3.91	0	0.49	0.49	0.49	0.49	0.98
C. coli - 15CC	1.95	0.49	0	0.49	0.49	0.49	0.65
C. jejuni - 17CJ	1.95	1.95	0.49	7.81	0.49	0.98	2.28
C. jejuni - 26CJ	0.98	0.49	0.49	0.49	0.98	0.98	0.74
C. jejuni - C1A8	0.49	0.98	0.49	0.49	0.49	0.49	0.57
C. jejuni - C1C3	0.98	0.49	0	0.49	0.49	3.91	1.06
C. coli - C1D3	0.49	0.49	0	3.91	0.49	0.49	0.98
C. coli - C1D9	0.98	0.98	0.49	0.98	0.49	0.98	0.82
C. coli - C1H6	0.98	0.98	0.49	0.49	0.49	0.49	0.65
C. jejuni - C3D9	0.98	0.98	0.49	0.49	0.49	0.49	0.65
C. coli - CC^{GR}	0.49	0.98	0.49	0.98	0.49	1.95	0.90

Maximum concentration allowing growth of *Salmonella* serotypes.							
Isolate	Maximum concentration						
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Avg
S. Kentucky	0	0	0.49	0	0	0	0.08
S. Heidelberg	0	0	0.49	0.49	0.98	0.98	0.49
S. Enteritidis	0.49	0	0.49	0.49	0.49	0.98	0.49
S. Typhimurium	0.49	0.49	0.49	0.49	1.95	1.95	0.98
S. Dublin	0	0	0.49	0.49	0.49	0.98	0.41
S. Infantis	0	0	0	0	0	0	0.0
S. Reading	0	0.49	0.98	0.49	0.98	0.98	0.65
S. Muenchen	0	0	0	0.49	0.98	0.98	0.41
S. Montevideo	0	0	1.95	0	0.49	0	0.41
S. Newport	0.49	0	0.49	0.49	0.98	0	0.41

Survival of *Salmonella* in filter sterilized carcass rinsate and CPC

Serotypeco	**ID #**	**Maximum Concentration Allowing Growth**			
		Rep 1	Rep 2	Rep 3	Avg
Kentucky	SQ0049	31.25	31.25	31.25	31.25
Heidelberg	SQ0050	125	125	31.25	93.75
Enteritidis	SQ0052	31.25	62.5	31.25	41.67
Typhimurium	SQ0066	250	250	62.5	187.50
Dublin	SQ0075	62.5	31.25	31.25	41.67
Infantis	SQ0078	125	31.25	62.5	72.92
Reading	SQ0088	250	125	125	166.67
Muenchen	SQ0090	125	125	250	166.67
Montevideo	SQ0181	125	31.25	62.5	72.92
Newport	SQ0187	125	125	62.5	104.17
Infantis	SQ0079	62.5	62.5	62.5	62.50
Kentucky	SQ0105	31.25	125	62.5	72.92
Infantis	SQ0108	62.5	125	62.5	83.33
Infantis	SQ0119	62.5	500	62.5	208.33
Kentucky	SQ0125	31.25	62.5	15.63	36.46
Kentucky	SQ0157	31.25	62.5	31.25	41.67
Kentucky	SQ0175	7.81	15.63	31.25	18.23
Infantis	SQ0218	62.5	125	62.5	83.33

CONCLUSION

Maximum CPC concentration growth in Buffered Peptone Water

Salmonella serotypes:	0.0 to 0.98 ppm
Campylobacter species:	0.57 to 2.28

Maximum CPC concentration growth in filter sterilized carcass rinsate:

Salmonella serotypes:	18 to 208 ppm

Evaluation of poultry water treatments during feed and water withdrawal on water usage and *Salmonella* prevalence in broilers

MATERIALS & METHODS

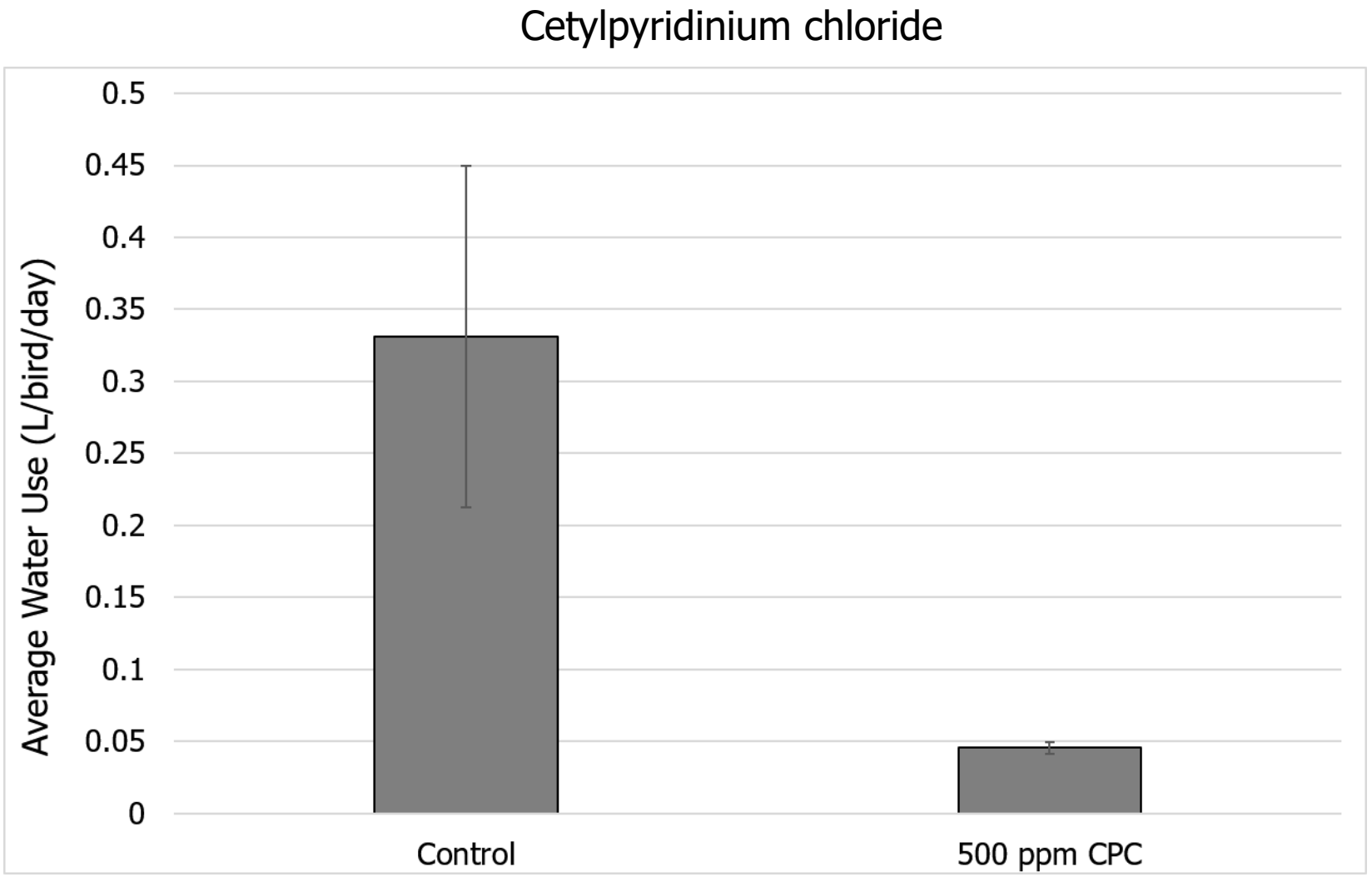
Water Treatments:

1. Control
2. Cetylpyridinium chloride 500 ppm
3. 50 ppm H_2O_2 + citric acid (pH adjusted to 5.0)
4. 50 ppm H2O2 + citric acid (pH adjusted to 6.2)
5. Sodium bisulfate (pH 3.2)

Feed	Water
0 h	0 h
6 h	0 h
12 h	6 h

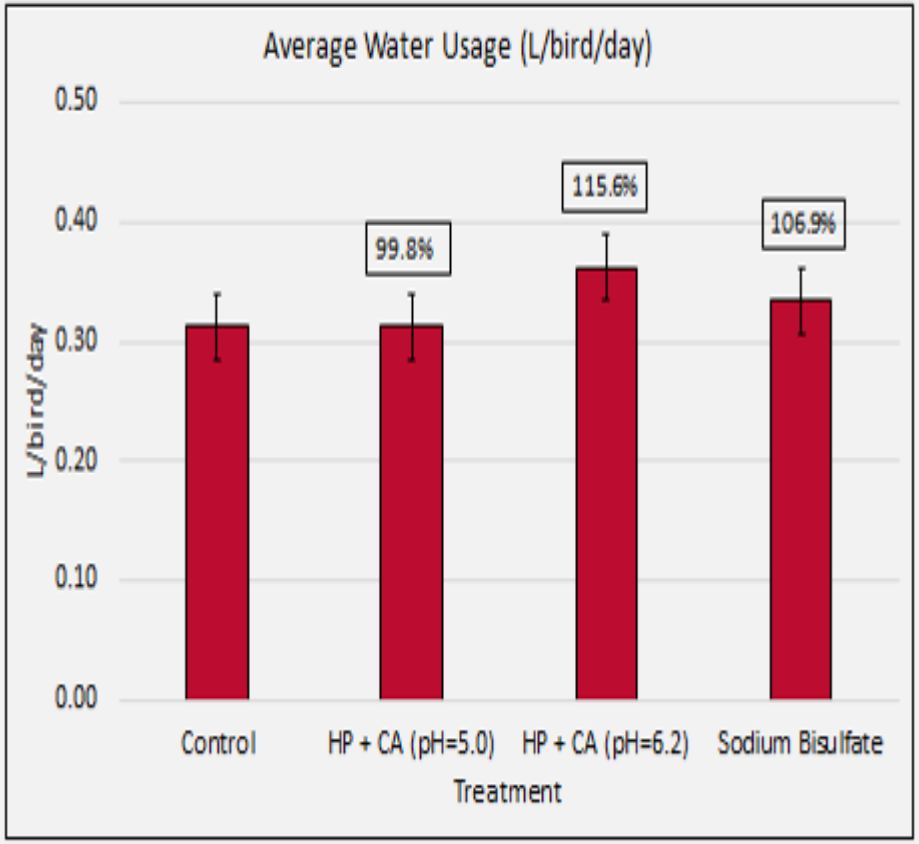
Broilers remained on treatment water for 2 days and then subjected to 1 of the 3 withdrawal schedules:

Water Usage before and during feed withdrawal

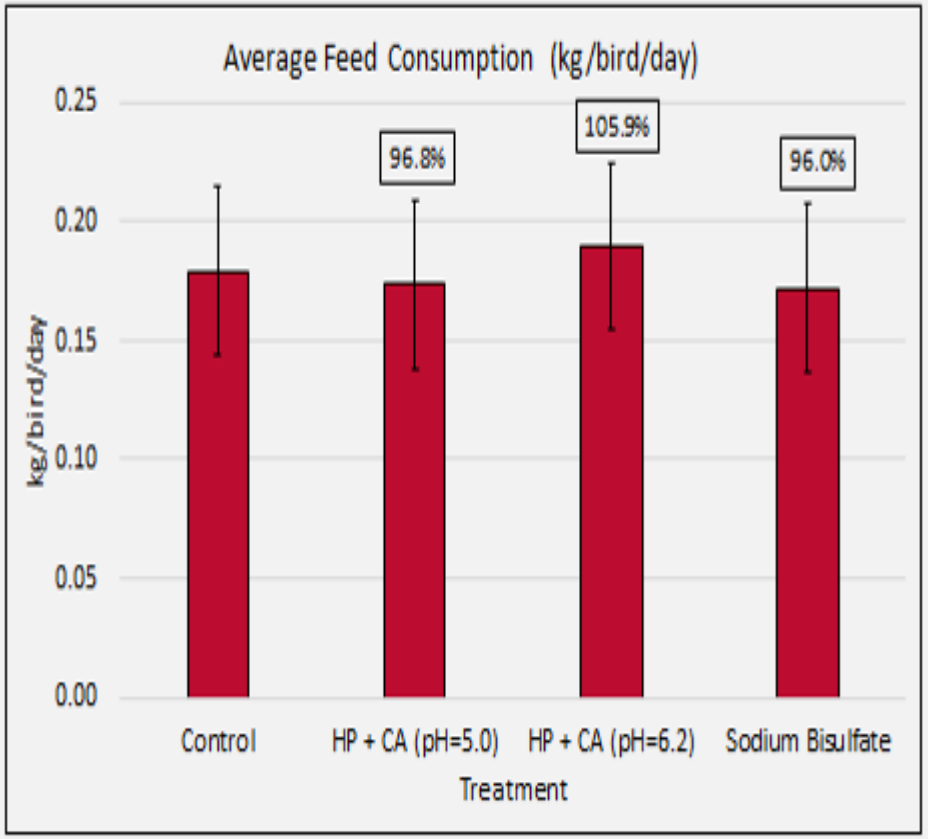


Water Usage and Feed Consumption before and during feed withdrawal

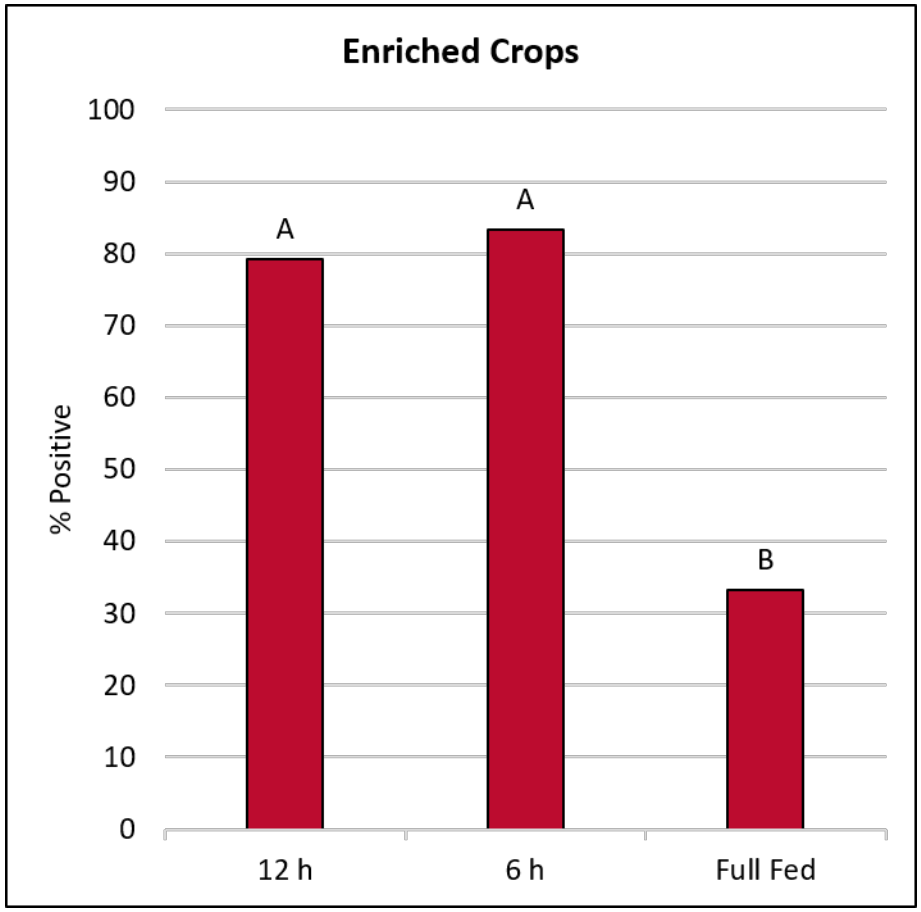
RESULTS

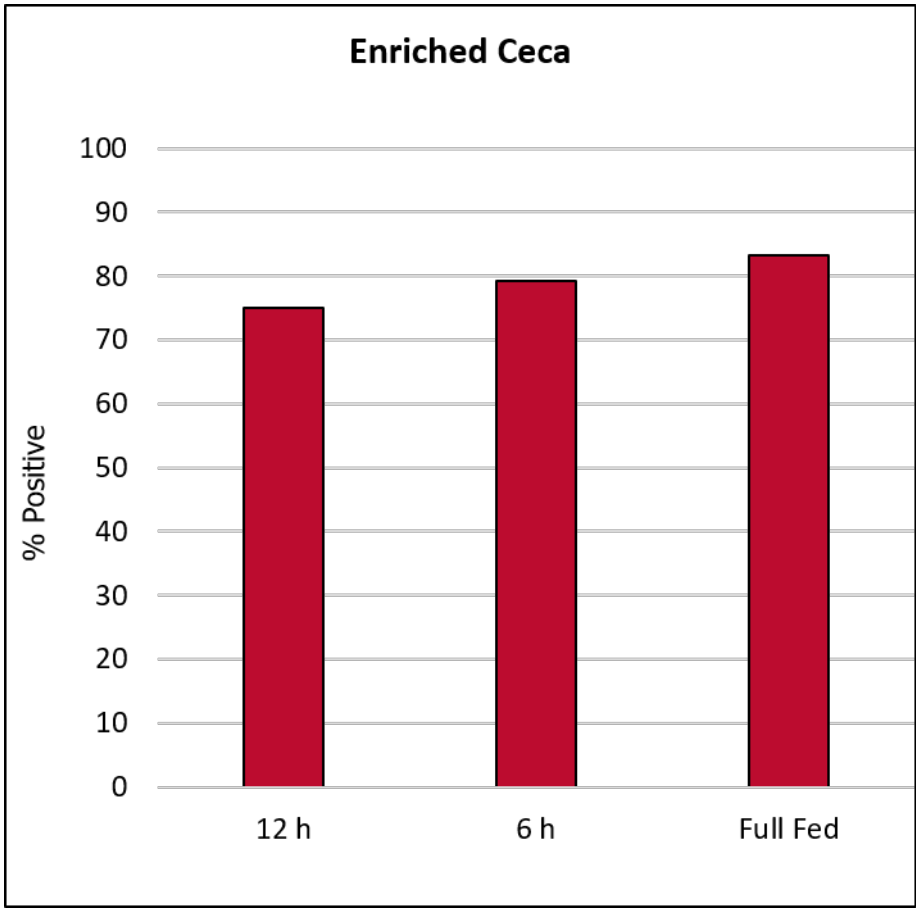


RESULTS



Salmonella prevalence in broilers subjected to poultry water treatments during feed and water withdrawal





CONCLUSION

Broilers will not drink water with Cetylpyridinium chloride at 500 ppm

No treatment impact on Crop or Cecal *Salmonella* recovery

- Cetylpyridinium chloride
- 50 ppm H2O2 + citric acid (pH adjusted to 5.0)
- 50 ppm H2O2 + citric acid (pH adjusted to 6.2)
- Sodium bisulfate (pH 3.2)

Mobile Bird Euthanasia Apparatus

For on farm euthanasia of large older birds (turkey and broiler breeders)

AVMA approved Euthanasia methods

Turkey Euthanasia Device

Koechner Euthanizing Device

Carbon Dioxide Euthanasia

Electrical Euthanasia

CONCLUSION

All successful Broilers, Laying hens, breeder Beltsville Small White Turkeys

Electrocution
Turkey Euthanasia Device
Koechner Euthanizing Device

Carbon Dioxide - challenge to stay under 40% CO_2 during first minute using a gas analyzer.

Animals 9:47, 2019, 1-8.
doi:10.3390/ani9020047

Questions?