Salmonella Roundtable Pre-Harvest Challenges and Solutions

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Salmonella and Broiler chickens

- Asymptomatic colonization
- *Salmonella* difficult to detect
  <1% of intestinal bacteria
- Intermittent shedding in feces
- Processed at 21 to 63 days of age

Recommended "Best practices"

- Vaccinating breeder flocks
- Hatchery *Salmonella*-free chicks
- Litter management / treatments
- Feed heat treatment & additives
- Water treatments
- Biosecurity = Humans, animals, insect

Vaccinated breeders - Sample broilers

Table 1. Comparison of Salmonella ceca total culture results from broilers challenged at d 1 by serovar in each column

Group[1]	*Salmonella* Kentucky	*Salmonella* Hadar	*Salmonella* Heidelberg	*Salmonella* Enteritidis
Vaccinated challenged	29/50 (58%)[2]	36/48 (75%)	40/48 (83%)	49/50 (98%)
Nonvaccinated challenged	43/50 (86%)	45/49 (92%)	46/49 (94%)	45/49 (92%)

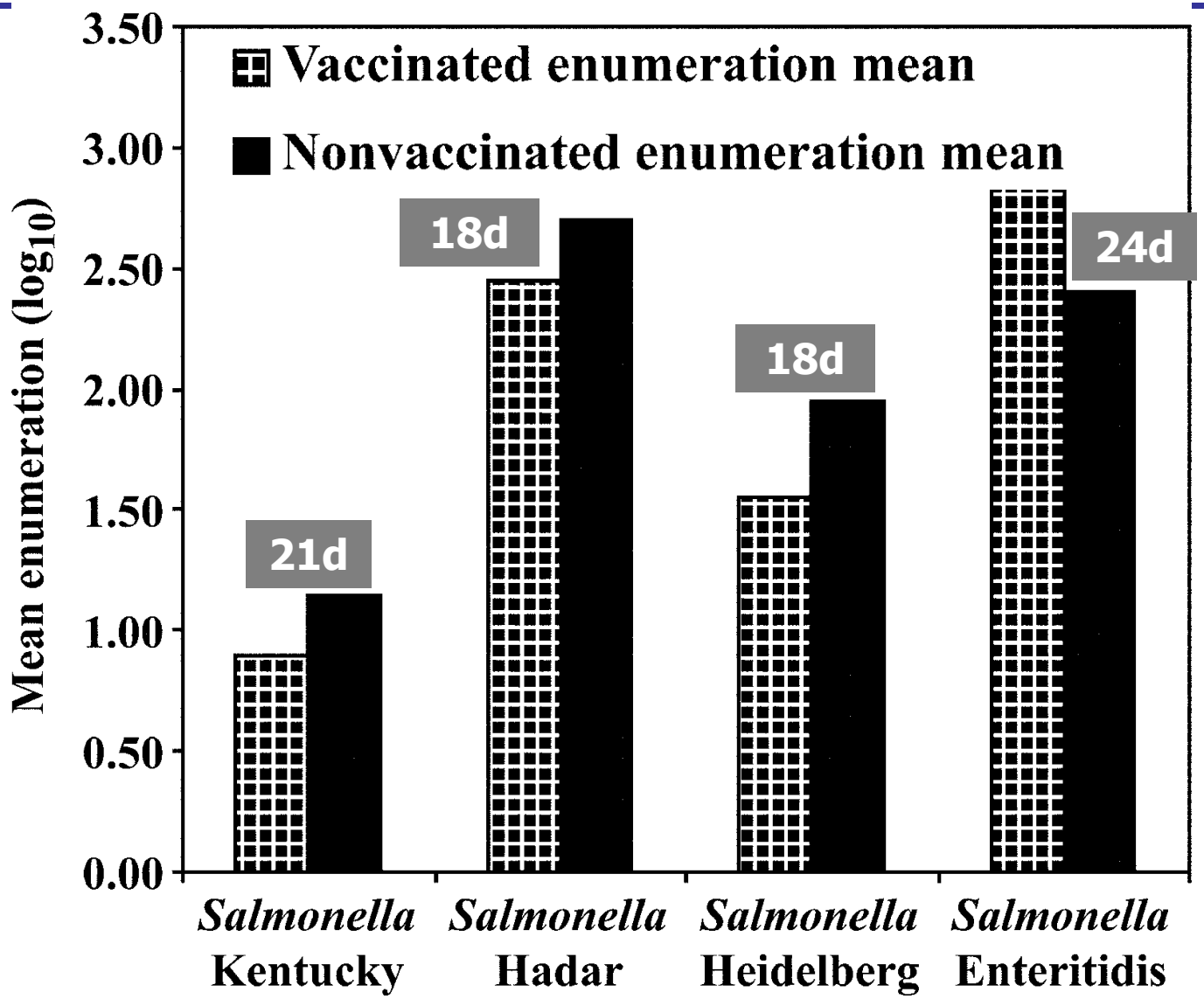
[1]No statistically significant differences between vaccinated or control.
[2]Culture positive number/total number cultured (percentage positive).

No statistically significant differences between vaccinated and control

At 1 d of age oral gavage with 1 × 10^6 cfu/broiler chick

doi.10.3382/japr.2007-00009

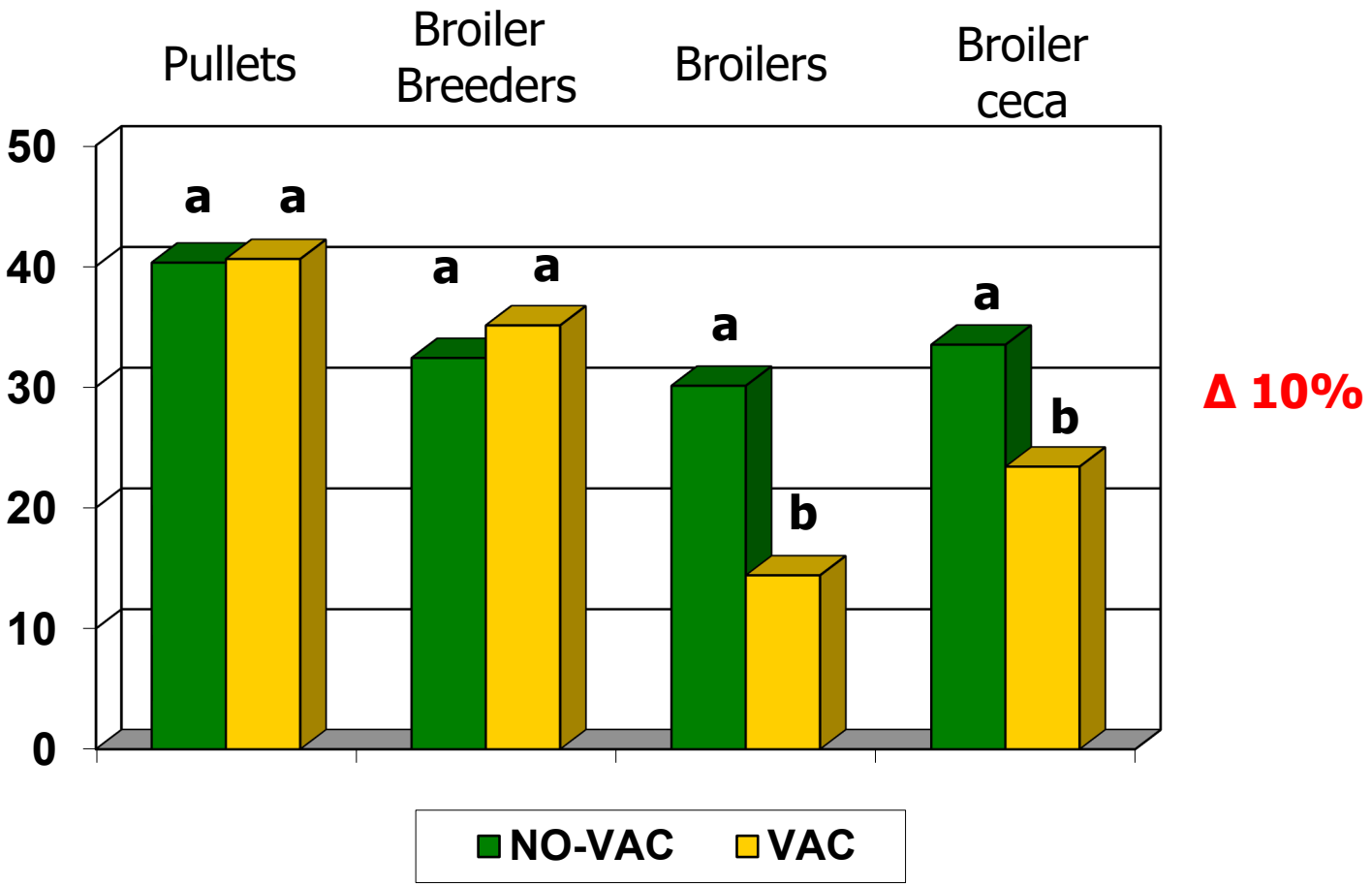
Vaccinate breeders – Broiler ceca



doi.10.3382/japr.2007-00009

Vaccinated Pullets 5-times

Litter *Salmonella* %+, Environmental *Salmonella*



Δ 10%

doi:10.1128/AEM.01320-10

4 Pullets flocks = 4 BB flocks x 4 Broiler flocks

Vaccinating breeder flocks

- Vaccinated + high challenge vs.

  No-Vaccinated + high challenge

  = **No significant benefit**

- Vaccinated + natural challenge vs.

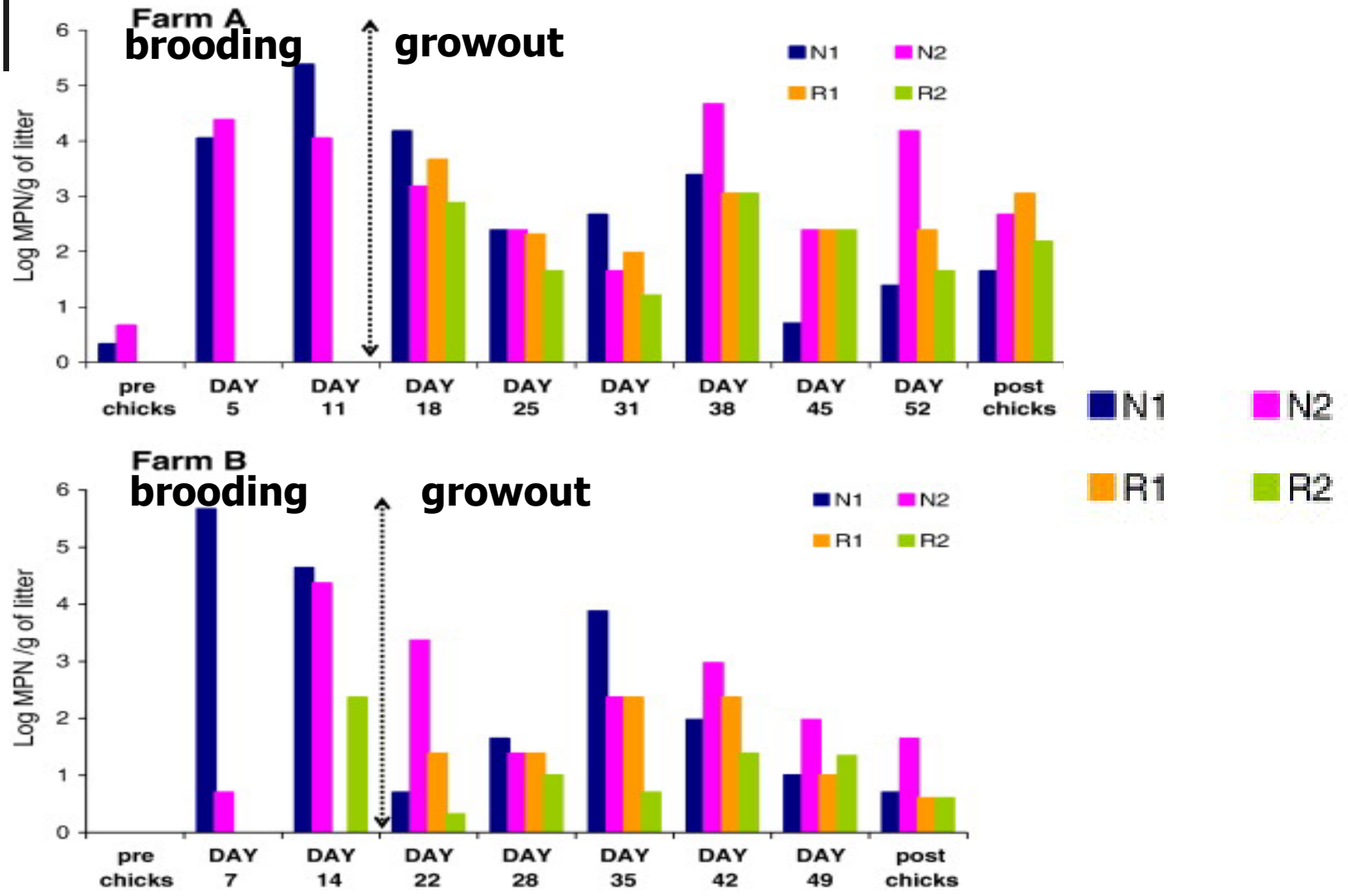
  No-Vaccinated + natural challenge

  = 23%+ vs. 33%+ = **Δ10 %** broiler ceca

Litter management / treatment

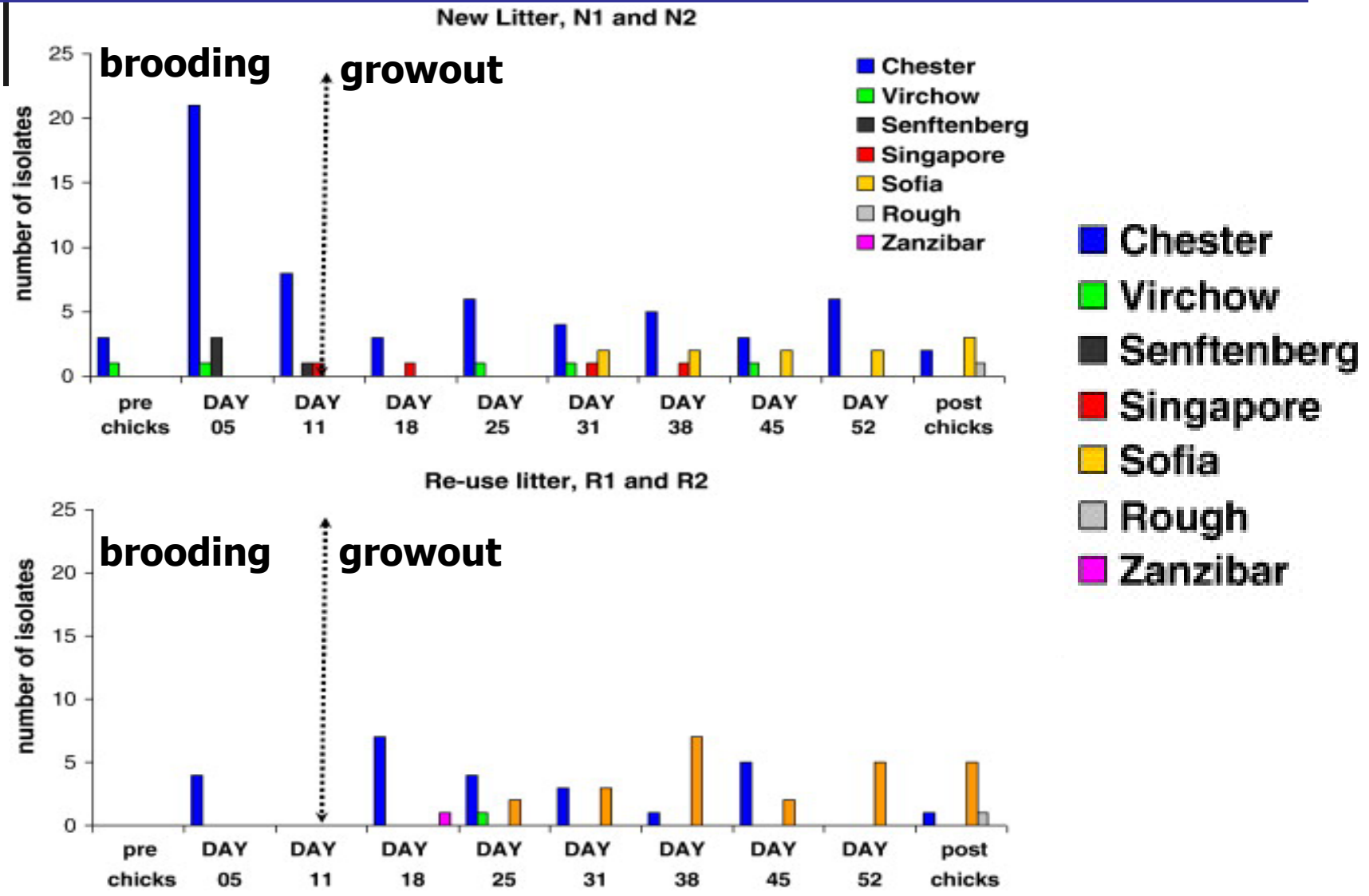
- New vs. Reused litter
- Between flocks till, windrow / compost
- Litter treatments for ammonia reduction during brooding (first 2 wks)
  Reduces mortality, foot/hock burns

Salmonella new & used litter



doi.org/10.1016/j.foodres.2011.06.014

Salmonella new & used litter



doi.org/10.1016/j.foodres.2011.06.014

Table 1. Number of positive and negative samples to *Salmonella* in reused litter.

Number of flocks reusing litter	1	2	3	4	5	6	7	Total
Salmonella positive	43[a]	19[b]	28	28	22	20	11	171
Salmonella negative	164	177	133	163	190	166	116	1109
Total	207	196	161	191	212	186	127	1280

[a, b] Different letters in the same line indicate statistical difference in Chi-Square ($p<0.05$).

No statistically significant differences after 2nd reuse

https://www.researchgate.net/publication/259928438_Presence_of_Salmonella_spp_in_reused_broiler_litter

Salmonella turkeys 3 vs.19 wk

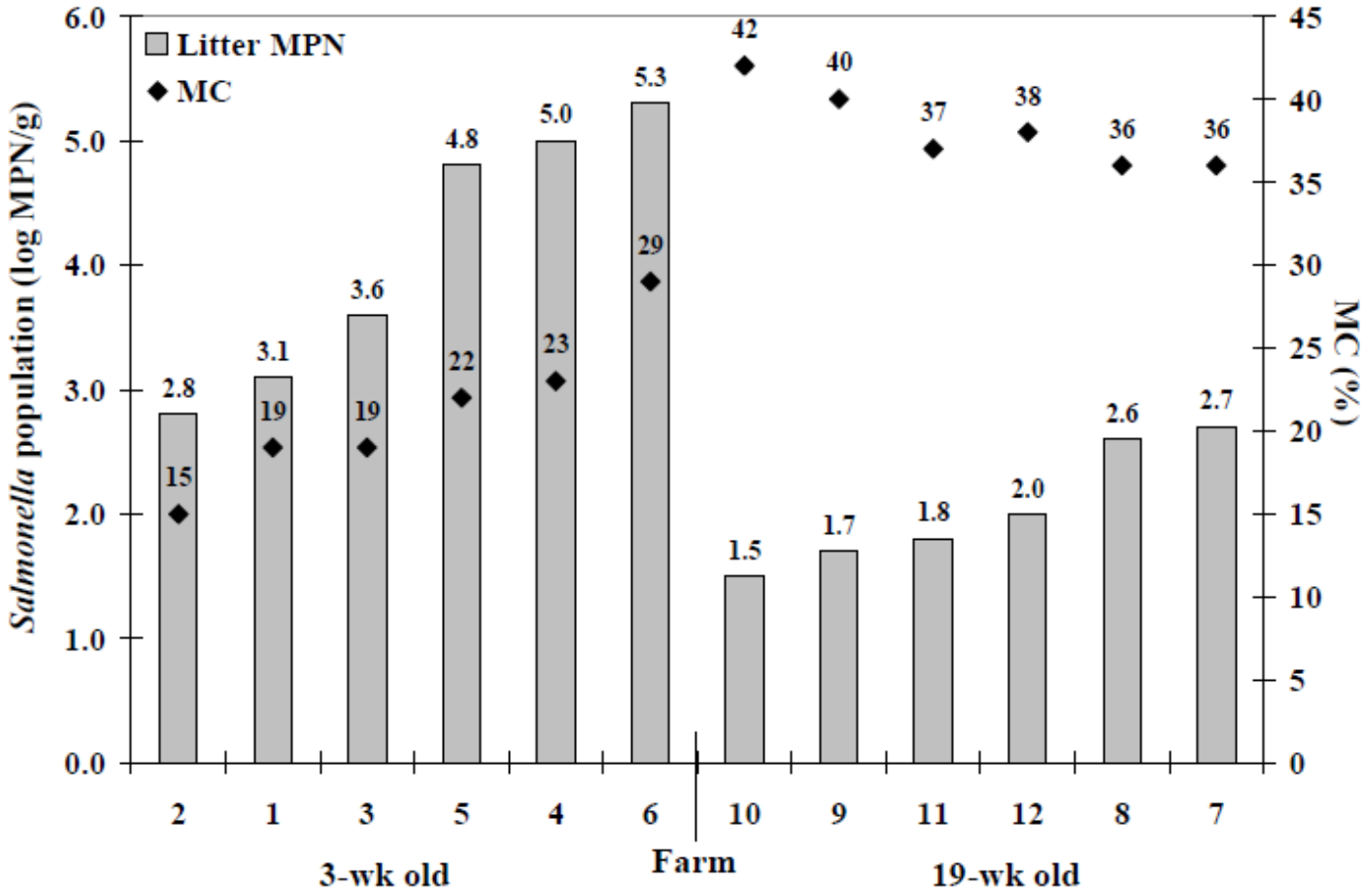


Figure 1. Litter moisture content (%) and *Salmonella* populations (log MPN/g) as influenced by farm and age (across seasons)

F. Santos PhD NCSU 2006

Reused Litter management goals

- Decrease ammonia volatilization
- Decrease darkling beetles
- Decrease moisture
- Decrease *Salmonella*, *Campylobacter* and *C. perfringens* (+coccidiosis = NE)

Litter windrowing / composting

- Down time between flocks 14-21 days
- 7-10 days temperature = 130˚F/54˚C
- Turn litter twice
- Beneficial to remove or break-up Wet & Caked litter

Litter windrowing / composting

Litter windrowing / composting

Flock litter management	Flock mortality	Beetle reduction*	Income improvement (per 1,000 birds)**
De-cake	6.2%	—	—
1st windrow	4.9%	76%	$23
2nd windrow	4.1%	76%	$89
3rd windrow	3.2%	~100%	$103

https://poultryhealthtoday.com/proper-windrowing-can-minimize-disease-benefit-environmental-management/

Litter windrowing / composting

Table 2. Bacterial levels that were inoculated into the respective treatments and the counts at 7 d postchallenge

Treatment	*Salmonella* (log_{10} cfu/g)	*Campylobacter* (log_{10} cfu/g)	*Clostridium perfringens*[1] (log_{10} cfu/g)
By treatment			
Initial levels	10.186[a]	11.575[a]	9.753[a]
Uncomposted	1.897[b]	0[b]	1.441[b]
Compost	0[c]	0[b]	0.833[b]
Probability			
Treatment	<0.001	<0.001	<0.001

[a–c] Letter differences signify that there was a difference in that column after a GLM was performed, followed by Tukey's multiple comparison test, at the *P*-value shown.

[1] Final *C. perfringens* is the total number of bacteria that were found in the sample. This number includes the *C. perfringens*

Uncomposted = on top of windrow, Composted = middle of pile, n=9

doi.10.3382/japr.2007-00051

Litter treatment acidifiers

- Acidifiers convert ammonia NH_3 to ammonium NH_4^+

- Sodium bisulfate $NaHSO_4$
- Aluminum sulfate $Al_2(SO_4)_3$
- Calcium sulfate $CaSO_4$
- Ferric sulfate $Fe_2(SO_4)_3$

Active for ~2 wk duration brooding

Brooding on new litter

Brooding on reused litter

Litter composite samples

- Litter grabs
- Drags swabs
- Socks
- Shoe covers
- ISODS

(**I**ntermittently **S**tepped **O**n **D**rag **S**wabs)

Litter


Drag swab

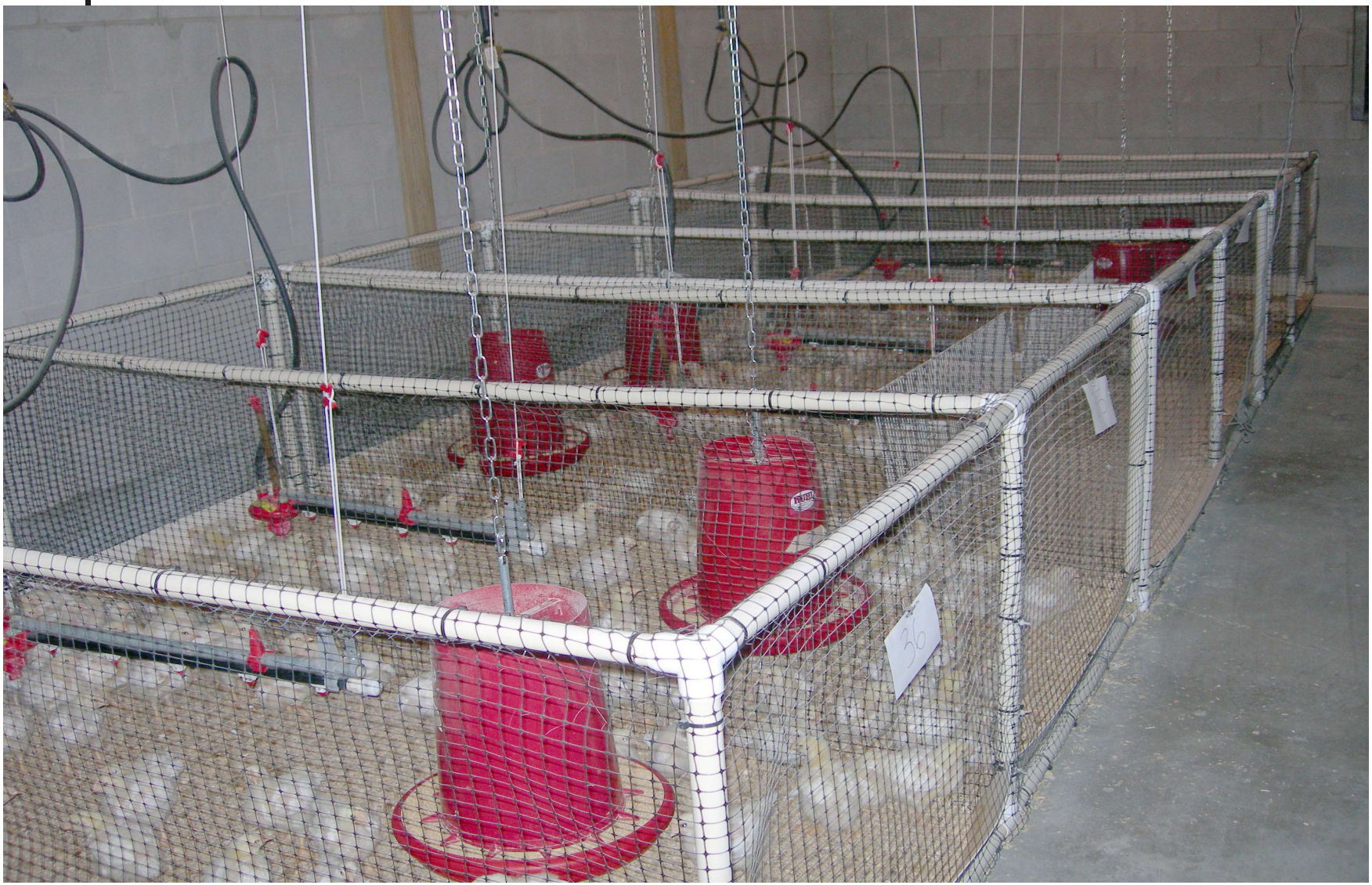

Sock


Shoe cover

Salmonella detection %-Positive

Sample	Exp 1	Exp 2
Sock	**53**	**67**
Drag Swab	19	44
Feces	17	--
Litter	11	--
ISODS	--	**69**

doi.org/10.1093/ps/86.1.21


36

Overall 1-7 wks *Salmonella+*

C	A	M	M	A	C
DS					DS
12 / 14	11 / 14	0 / 14	2 / 14	9 / 14	11 / 14
ISODS					ISODS
13 / 14	12 / 14	4 / 14	5 / 14	11 / 14	13 / 14
Δ 1	1	4	3	2	2

doi.org/10.1093/ps/86.1.21

Litter Sampling

- *Salmonella* detection - **ISODS**

(**I**ntermittently **S**tepped **O**n **D**rag **S**wabs)

- 20% increase in *Salmonella* detection from litter with ISODS

"fewer false negatives"

doi.org/10.1093/ps/86.1.21

Flock Sampling Methods

Non-Invasive:

- Cloacal Swabs (individual)
- Feces (individual or composite)
- Drag swabs (composite)

Invasive:

- Ceca (individual)
- Spleen (individual)

Salmonella BB-Hens - Pen 8

Hen	Cloaca Swabs	Ceca	Spleen
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	-	-	-
8	-	-	-
9	-	-	-

Montiel 2012 Poultry Science

Salmonella BB-Hens - Pen 6

Hen	Cloaca Swabs	Ceca	Spleen
1	-	+	+
2	+	+	+
3	-	+	+
4	-	+	+
5	-	-	-
6	+	+	+
7	-	+	+
8	-	-	-
9	-	+	+

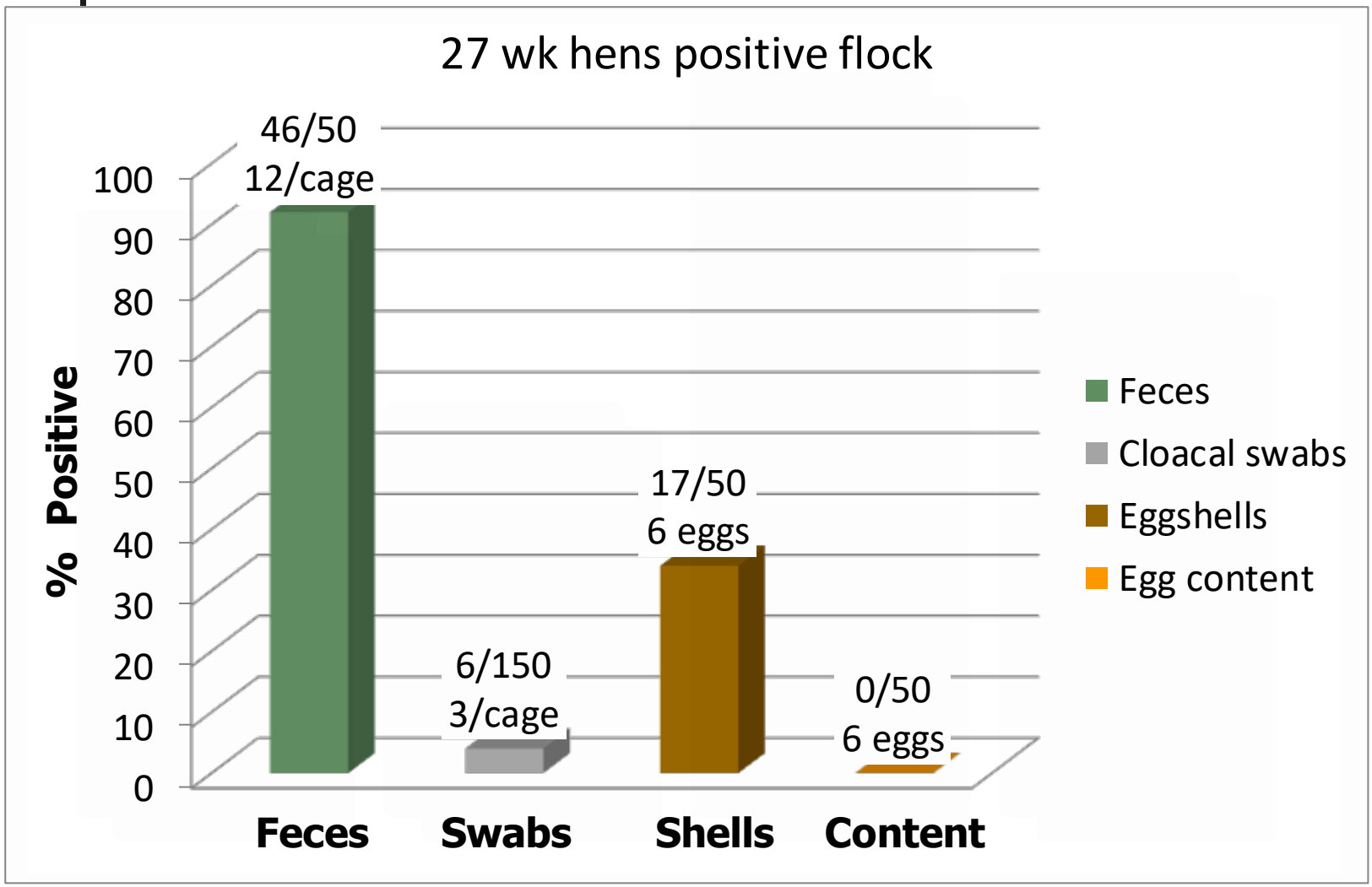
Montiel 2012 Poultry Science

Salmonella BB-Hens - Pen 5

Hen	Cloaca Swabs	Ceca	Spleen
1	-	-	-
2	-	-	-
3	-	+	+
4	-	+	+
5	-	+	+
6	-	+	+
7	-	-	+
8	-	+	+
9	-	-	-

Montiel 2012 Poultry Science

Salmonella caged egg laying hens



doi.10.3382/ps.2010-01104

Recommendations

- Use sampling methods other than cloacal swabbing
- Feces-litter / ceca / spleen
- Composite or Pooled > Individual

Interventions

- Competitive exclusion non-pathogenic
- Feed & Water Pre-and probiotics
- Bacteriophages or bacteriocins
- Antimicrobial compounds
  - Sodium chlorate, Essential oils, Metals (e.g., zinc, copper)

Pro-biotic v. Pre-biotic?

- Probiotic = Live bacterial = Yogurt
- Prebiotic = substrates for alimentary tract bacteria: fiber, chemicals, nutrients

Organic Acids & Fatty Acids = chemicals

Prebiotic - food sources

Organic acids mode of action

Lipid soluble, diffusion through cell membrane of Gram- bacteria

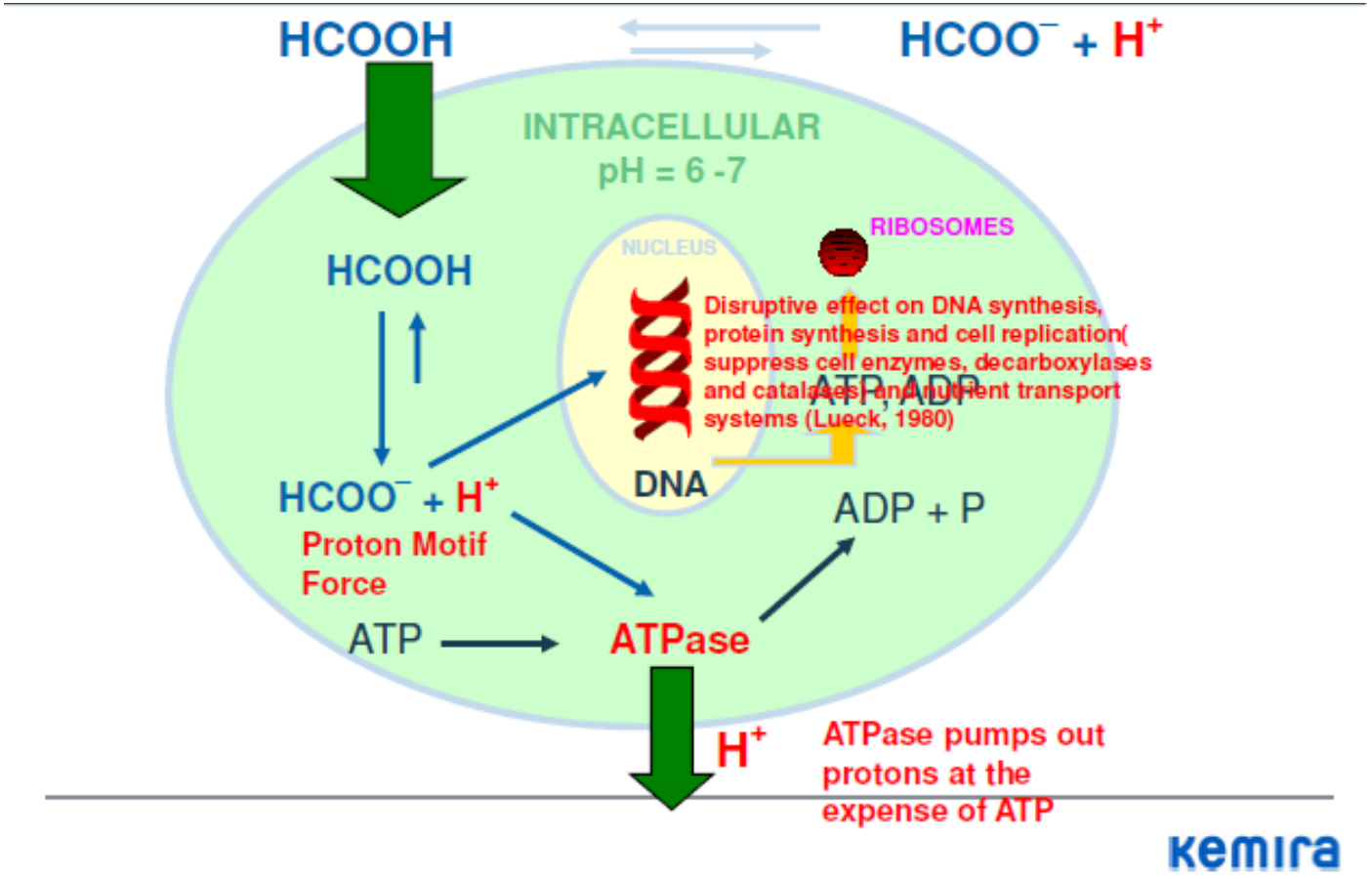
Dissociate H+ lower intracellular pH 3-4

Lower pH disrupt cell function -> death

Gram- bacteria are able to metabolize medium and long chain FA

Organic acids mode of action

Bactericidal effects of Organic Acids in the gastric environment **pH 3 to 4**



Gram +/- Jejunum and Ceca

TABLE 1. Bacterial genera detected in both the ileal and cecal 16S rDNA libraries

Group (% of total)	Genus	% of genus in:[a]	
		Ileum	Cecum
Low G+C, gram positive (ileum, 94.18; cecum, 76.9)	*Lactobacillus*	67.59	7.75
	Weisella	1.05	0.48
	Clostridium	9.69	39.26
	Ruminococcus	0.44	16.48
	Eubacterium	0.73	9.85
	Bacillus	0.67	1.45
	Staphylococcus	0.95	0
	Streptococcus	6.63	0.65
	Enterococcus	6.43	0.97
High G+C gram positive (ileum, 0.92; cecum, 13.89)	*Fusobacterium*	0.73	13.89
	Bifidobacterium	0.19	0
Proteobacteria (gram negative) (ileum, 2.28; cecum, 2.75)	*Ochrobacterium*	0.18	0.81
	Alcaligenes	0.88	0.65
	Escherichia	0.35	1.29
	Campylobacter	0.88	0
Cytophaga/Flexibacter/Bacteroides (ileum, 0.6; cecum, 5.19)	*Flavobacterium*	0	0.16
	Bacteroides	0.60	5.01

[a] A total of 614 and 616 sequences were analyzed from the ileum and cecum, respectively.

doi.10.1128/AEM.69.11.6816–6824.2003

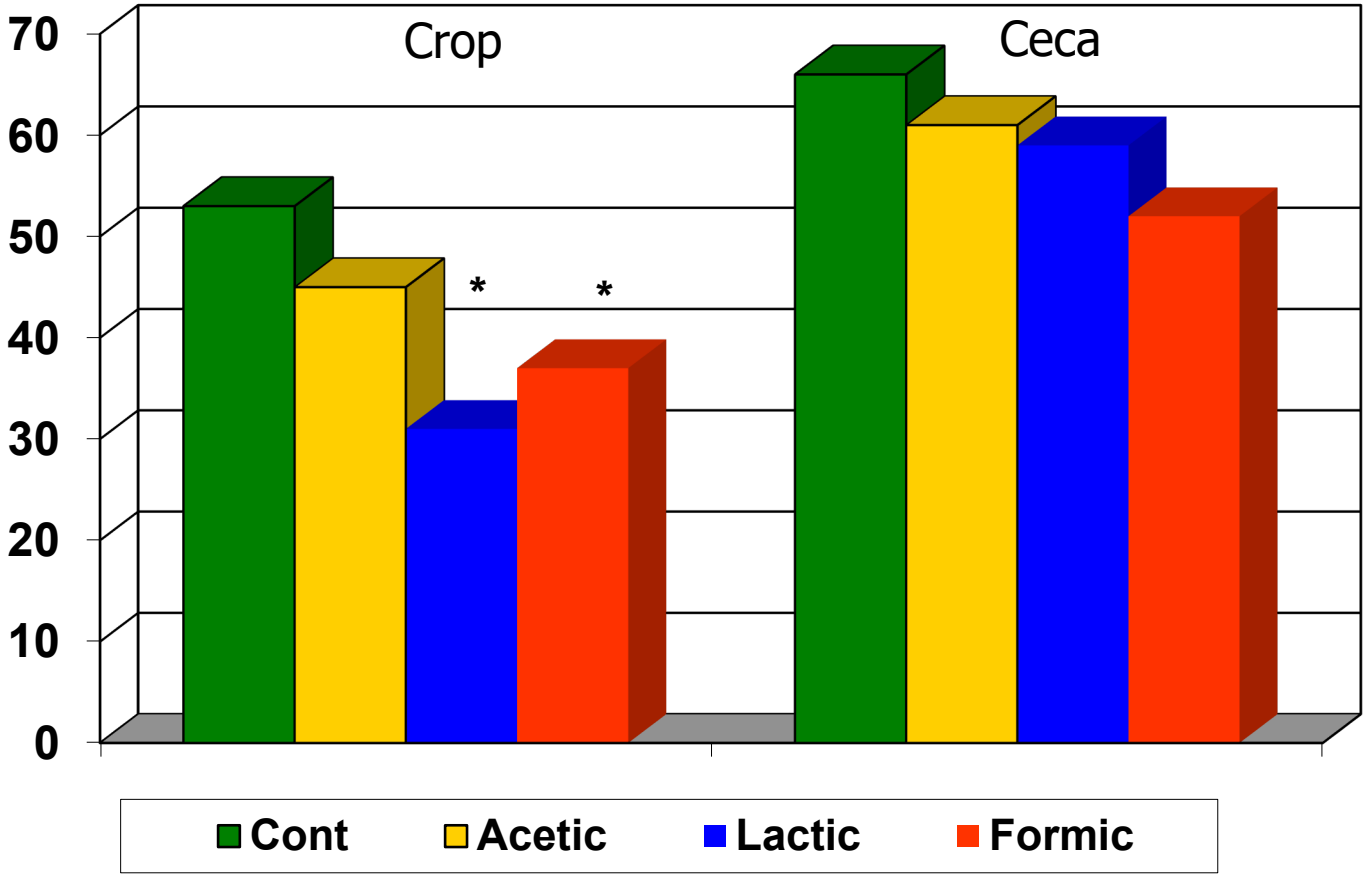
Gram +/- Jejunum and Ceca

Gram stain	Jejunum	Ceca
Positive +	**95%**	**91%**
Negative -	<3%	<8%

doi.10.1128/AEM.69.11.6816–6824.2003

Acetic, Lactic, Formic 0.5% - Water

Salmonella % positive in Crop and Ceca

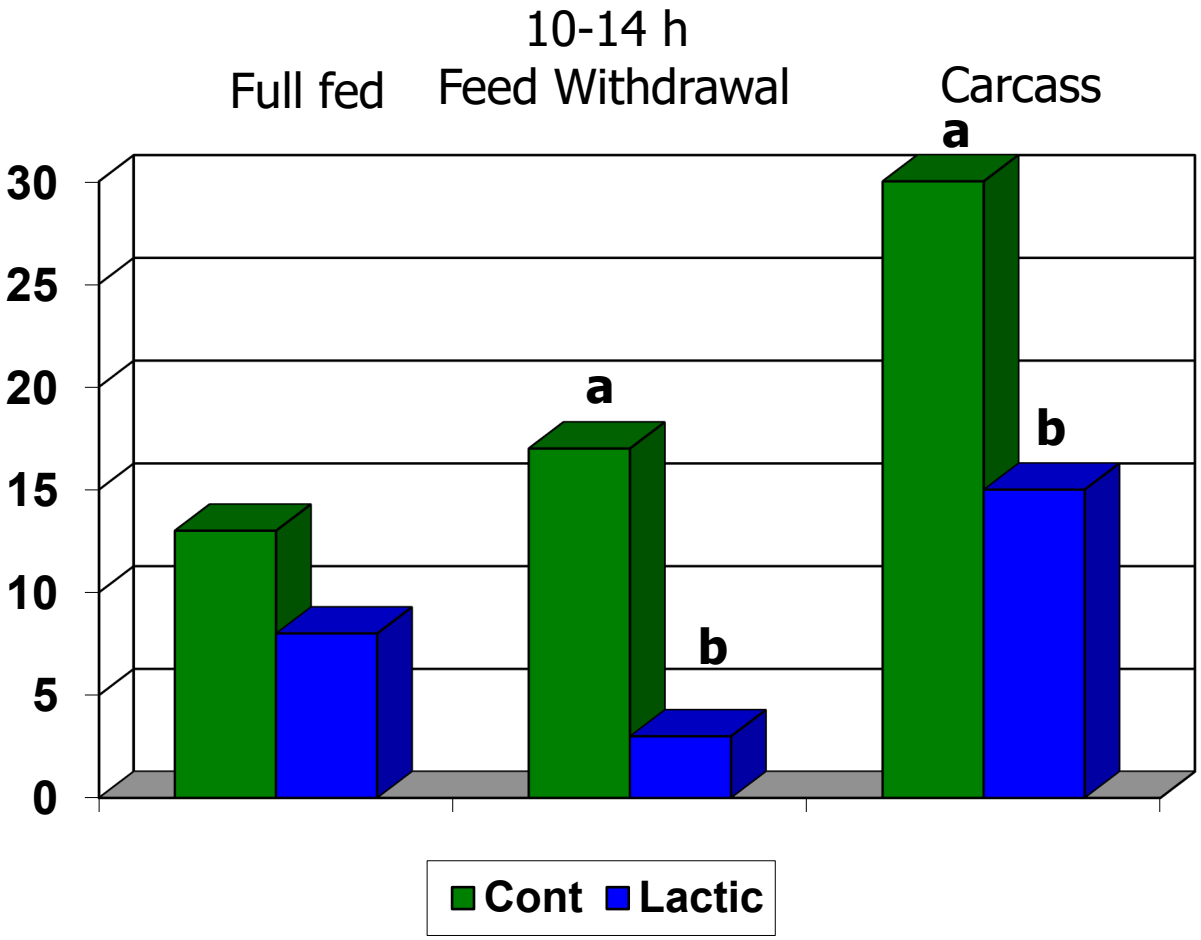


doi.org/10.1093/ps/80.3.278

Challenged 10^8 cfu 35 & 41d, Sampled 42d

Lactic Acid C3 0.44% - Water

Salmonella % positive in Crop and Carcass rinse

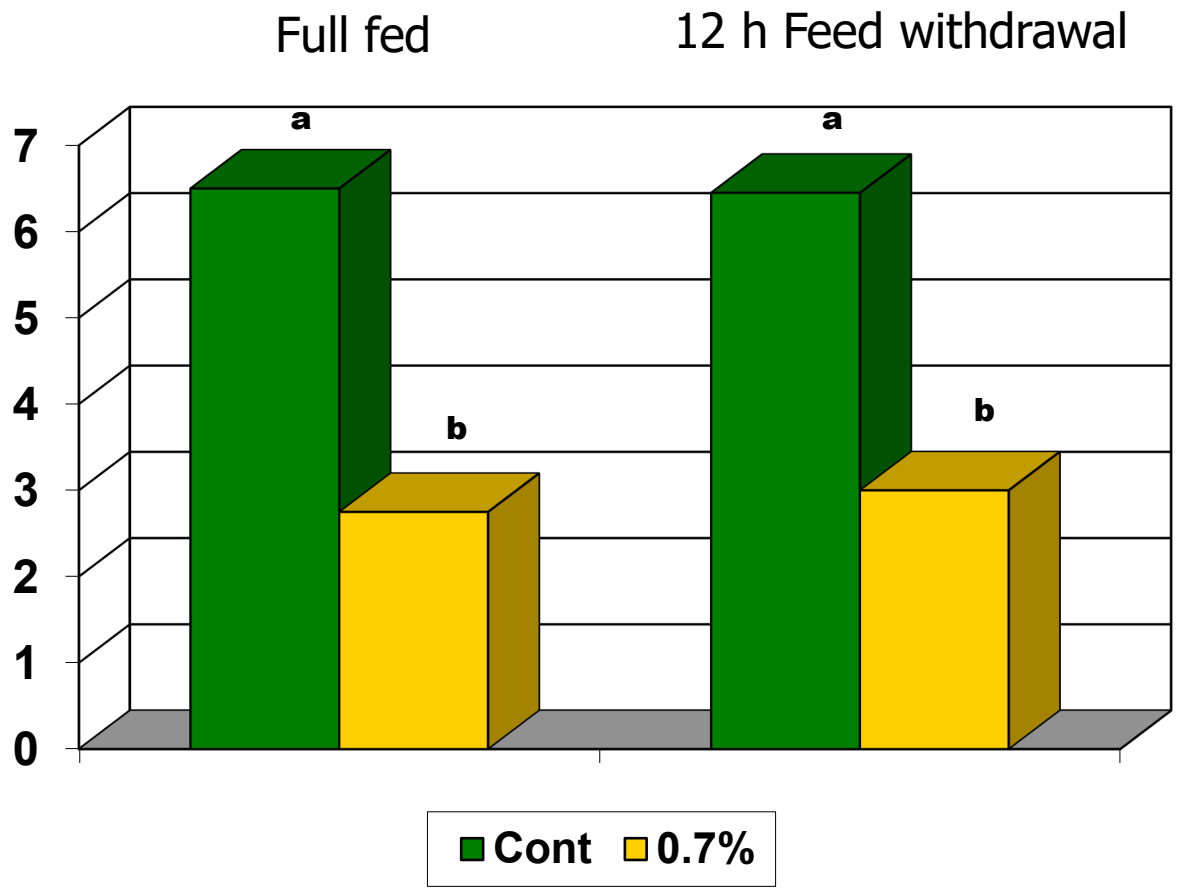


doi.org/10.1093/ps/80.3.278

Natural challenged, Acid during feed withdrawal

Caprylic Acid C8 0.7% - Feed

Cecal *Campylobacter* $\log_{10}$cfu/g - last 3 days

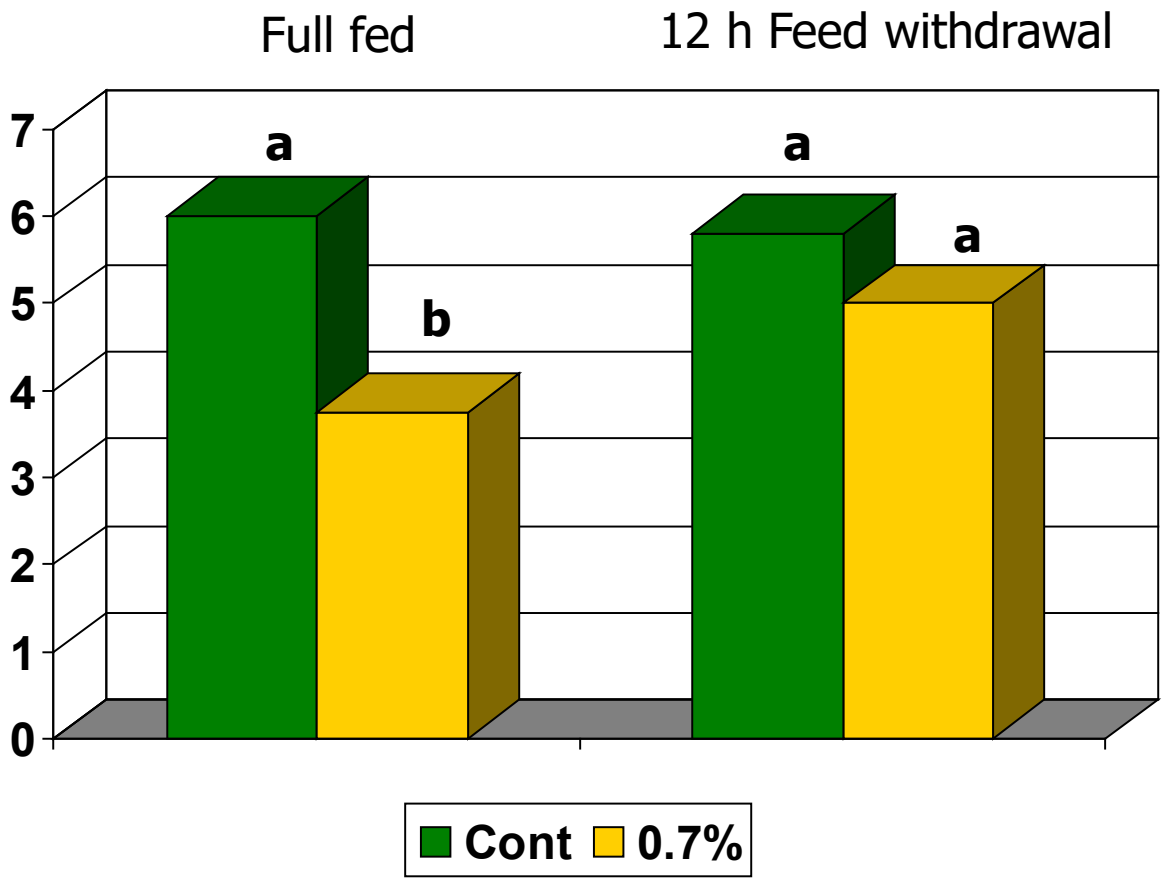


doi.10.1128/AEM.02528-07

Challenged 21d, Sampled 42d

Caprylic Acid C8 0.7% - Feed

Cecal *Campylobacter* $\log_{10}$cfu/g - last 7 days



doi.10.3382/ps.2008-00228

Challenged 21d, Sampled 42d

Formic + Lauric feed - Broilers

- Formic C1
- Formic C1+ Lauric C12 @5kg/ton

- Male broiler chicks Cobb / Ross
- Challenge 3 / 33 chicks / pen
- Unchallenged adjacent pens

doi.org/10.3382/ps/pex289

Experimental design - Broilers

- Litter - weekly ISODS
- Ceca at weeks 3 and 6
- Process week 6, after 10h FW
  - WCR & WCE

doi.org/10.3382/ps/pex289

WCE 430 ml of BPW, 30 ml rinsate for WCR

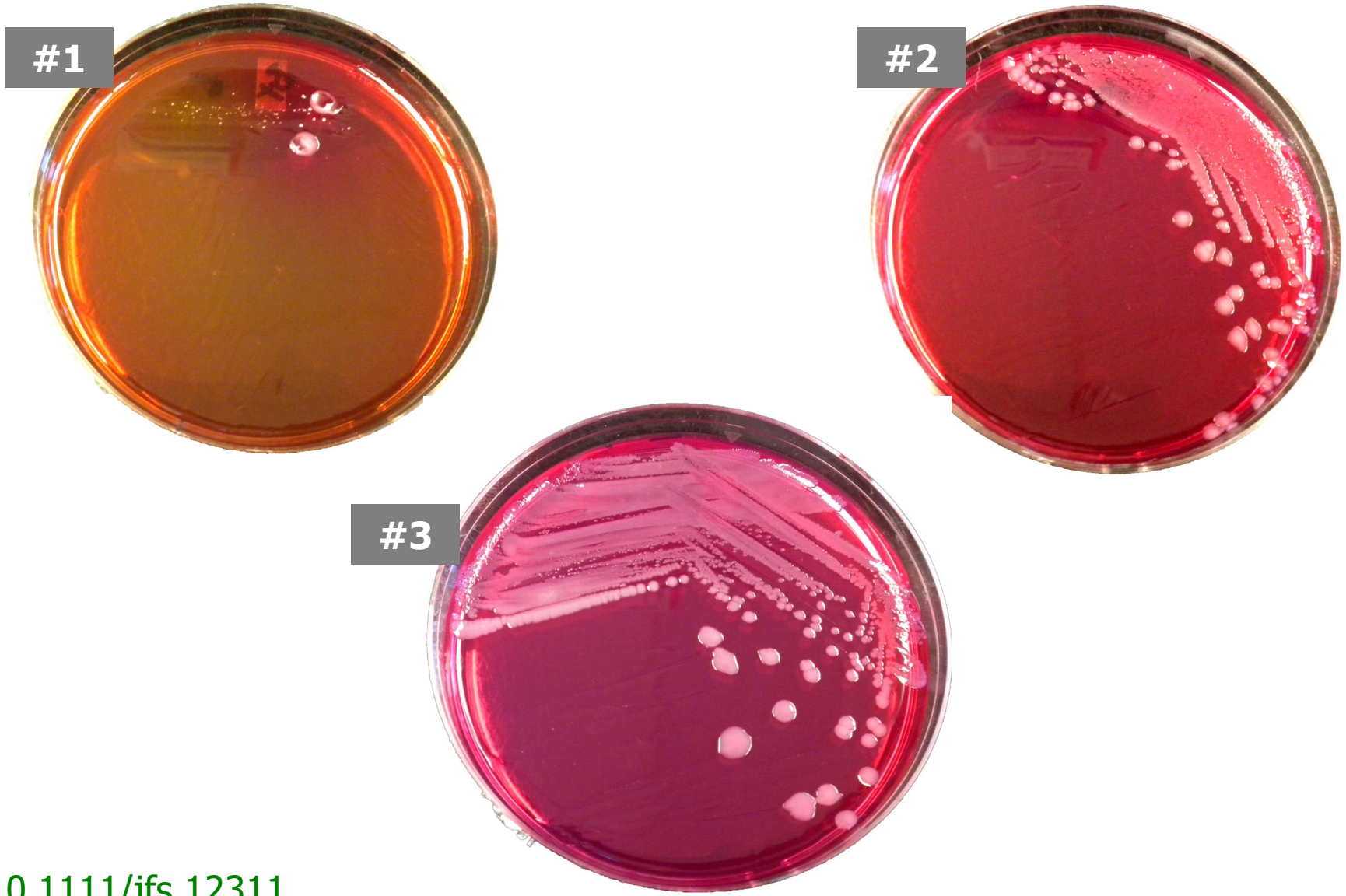
Whole Carcass Rinse

30 ml rinsate BPW for WCR

Whole Carcass Enrichment

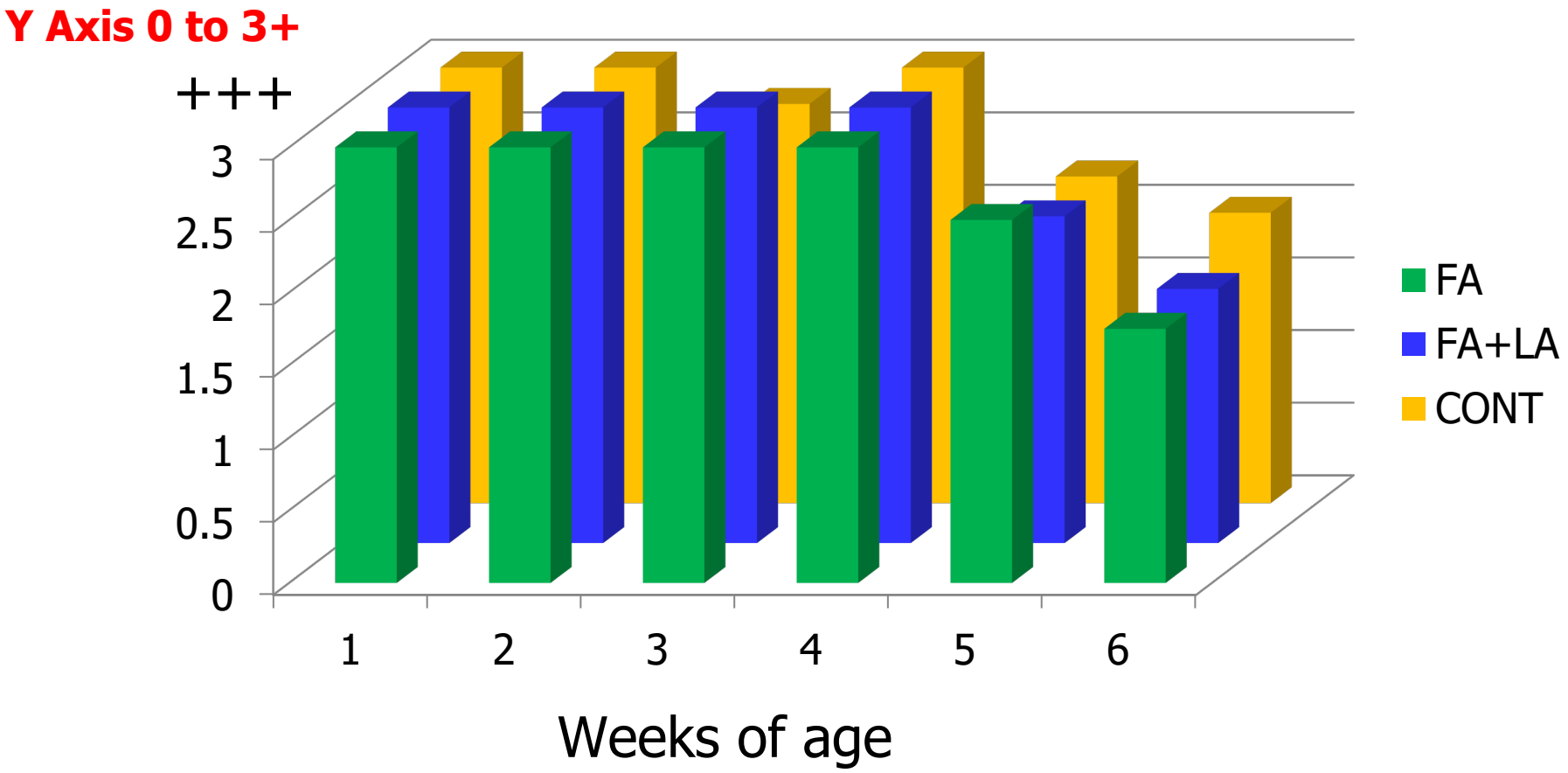
400 ml of BPW for WCE

Salmonella on BGS plates



doi.10.1111/jfs.12311

Litter *Salmonella* challenged pens



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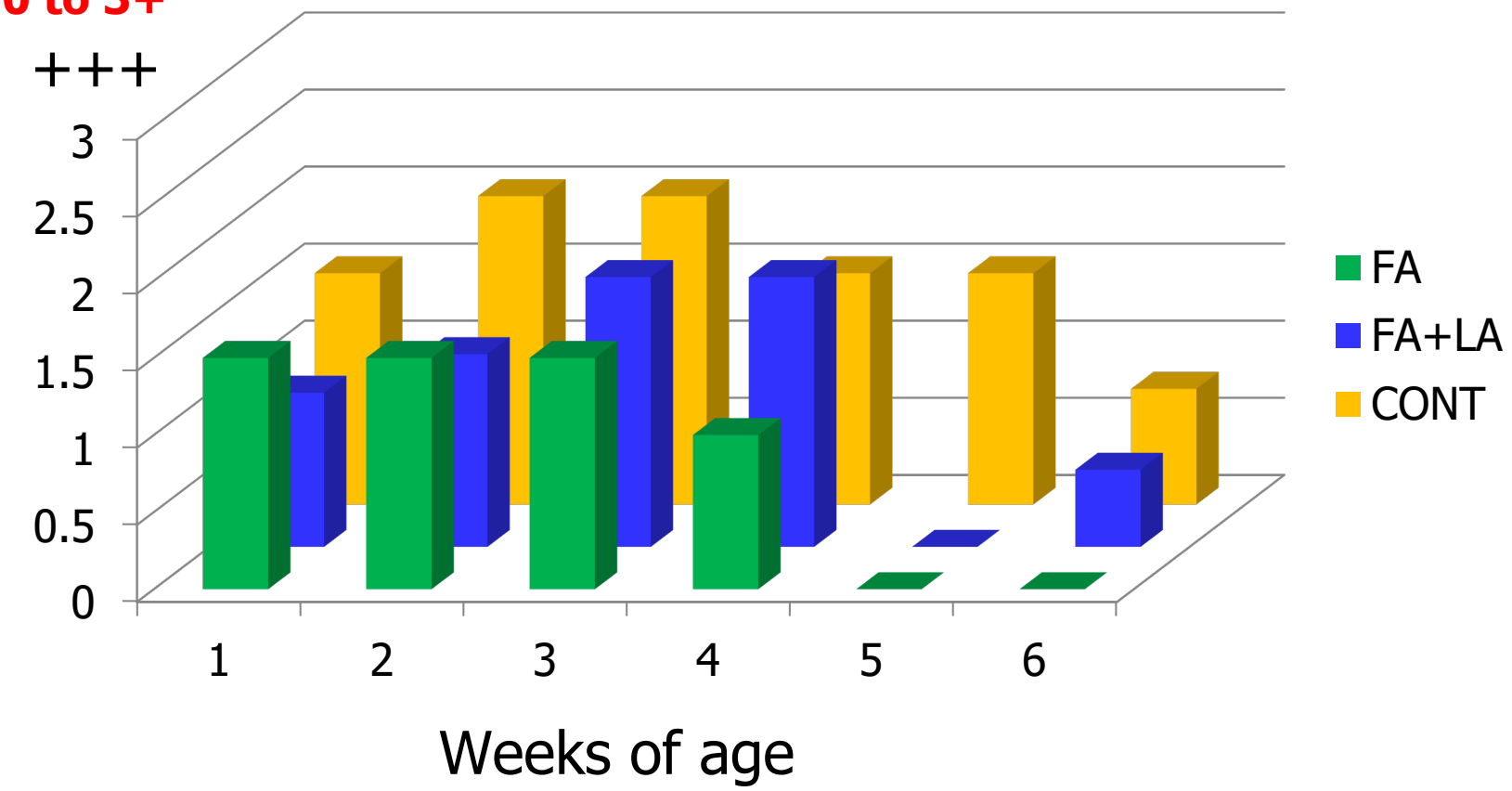
Litter *Salmonella* adjacent pens

Y Axis 0 to 3+



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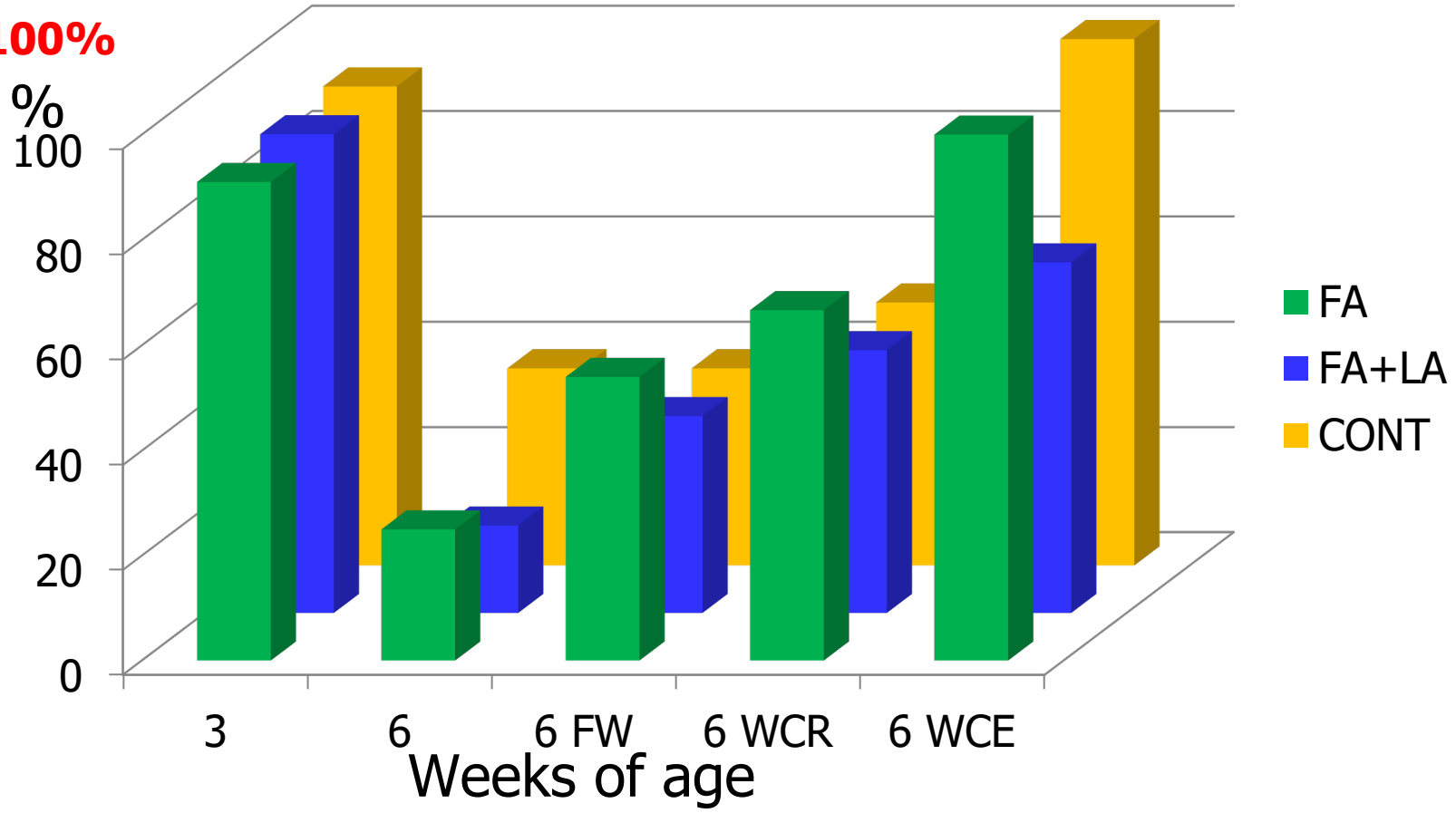
Ceca *Salmonella* Challenged pens

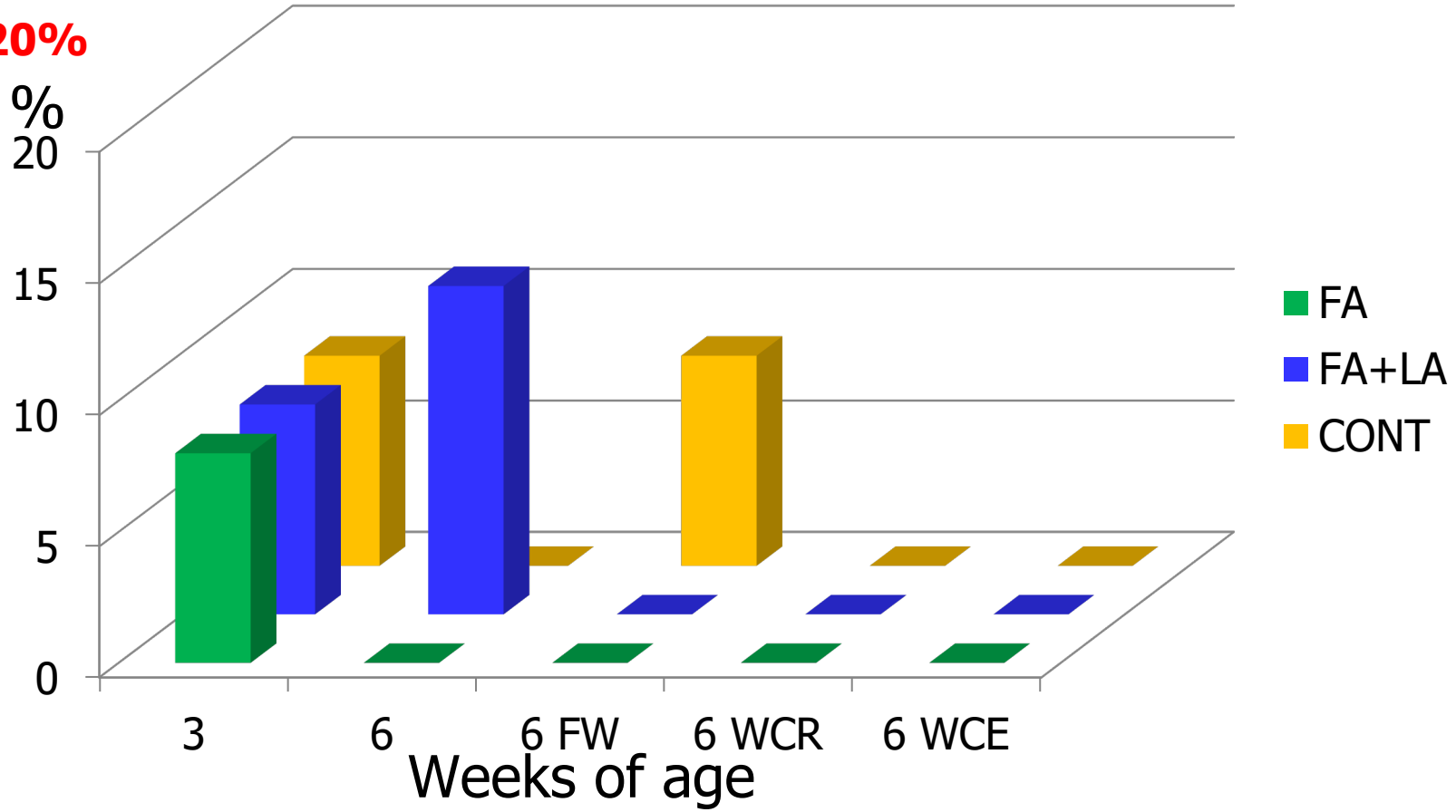
Y Axis 100%



doi.org/10.3382/ps/pex289

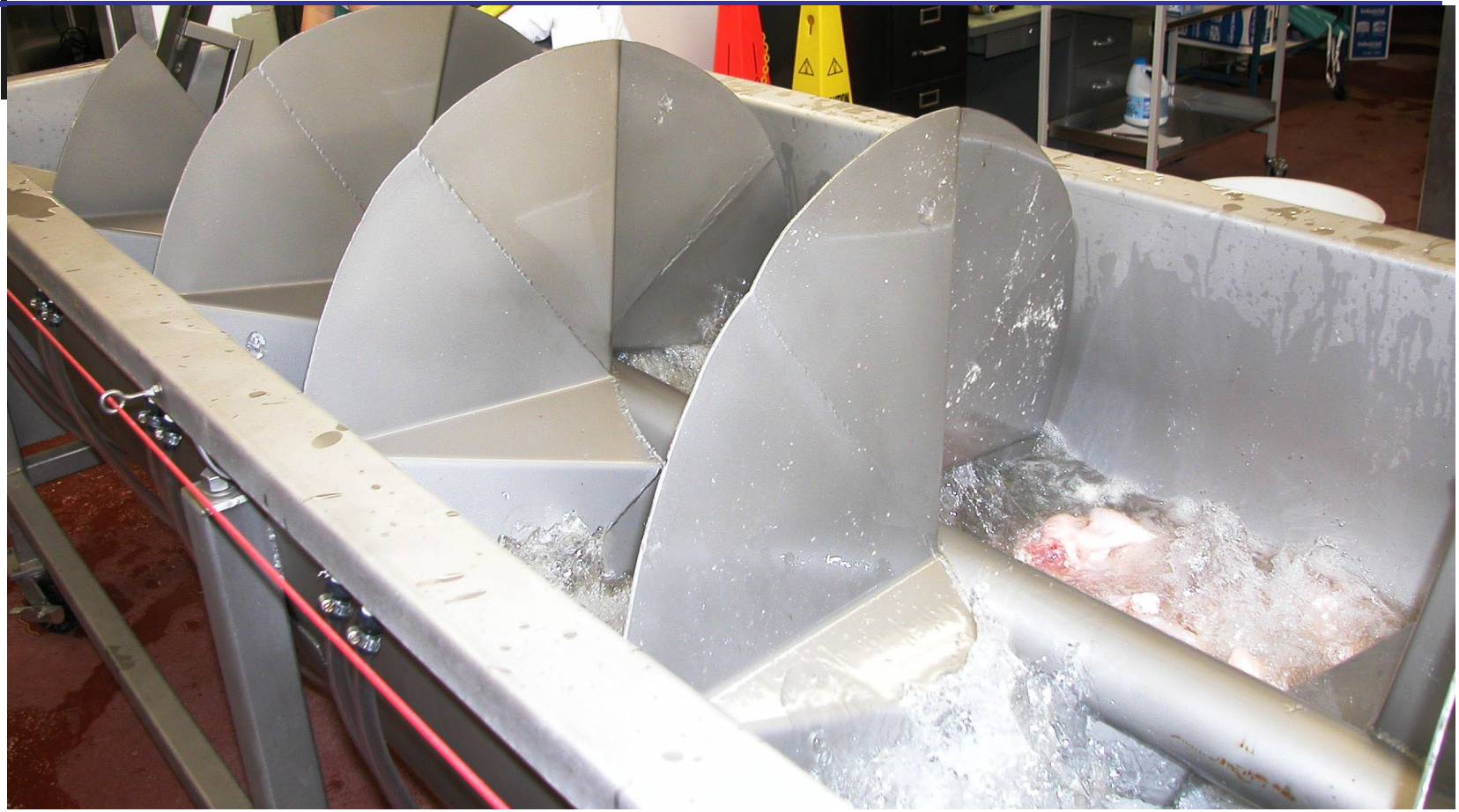
Ceca *Salmonella* adjacent pens

Y Axis 20%



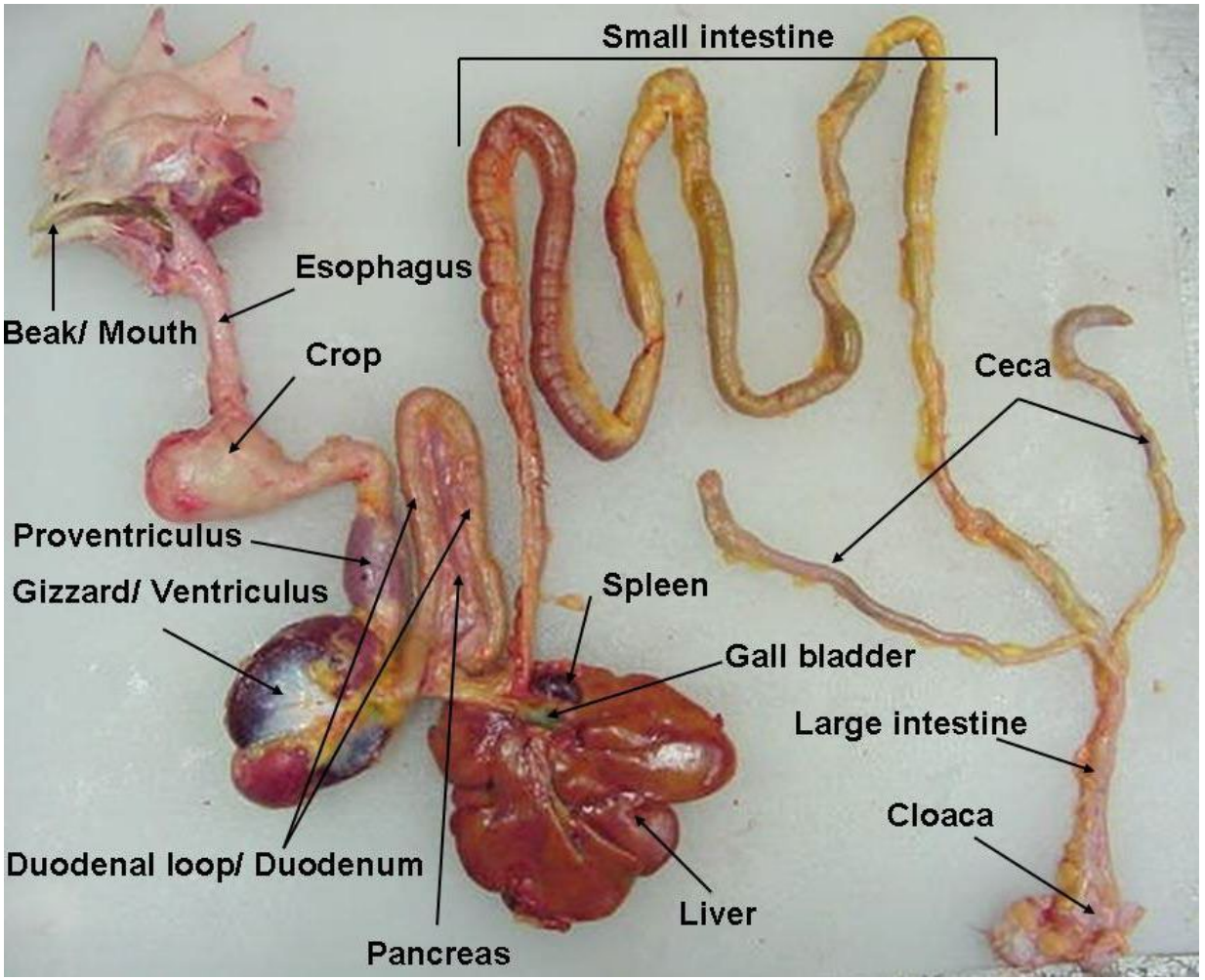
doi.org/10.3382/ps/pex289

Immersion chilling antimicrobial



The Main Critical Control Point!

Alimentary tract chicken



Normal pH of Alimentary tract

Crop	4.84
Proventriculus	3.48
Gizzard	2.56
Duodenum	5.46
Jejunum	6.00
Ileum	6.24

Organic acids mode of action

Micro-organisms are not tolerant to low pH

pH	E.coli	Salmonella	Clostridium
6.4	++++	++++	++++
6.0	+++	+++	++++
5.8	++	++	++++
5.4	+	+	+++
5.0	-	+-	++
4.5	-	-	+
4.0	-	-	-

kemira

pH of tract Butyric acid 0.6%

Crop	4.84	4.01*
Proventriculus	3.48	3.02*
Gizzard	2.56	2.14*
Duodenum	5.46	5.19*
Jejunum	6.00	5.82
Ileum	6.24	6.16

Conclusion

- Adding organic acids can lower the pH of alimentary tract contents from the Crop to Duodenum

- But minimal impact on the pH of contents of the Jejunum, Ileum, Ceca, or Colon

Prebiotic during feed withdrawal

Will carbohydrate-based cocktails decrease the population of *Salmonella* and *Campylobacter* in the crop of broiler chickens subjected to feed withdrawal?

doi.org/10.1093/ps/81.6.780

Experimental design - Cocktail

Day 1

- 6-week commercial broilers
- Challenge *Salmonella* / *Campylobacter*

Day 2

- Cocktail provided 4 hours with feed
- Cocktail provided during 12-hour feed withdrawal

doi.org/10.1093/ps/81.6.780

Experimental design - Cocktail

Day 3

- Process through defeathering:
  - Crop and Ceca - Weight and pH
  - Lactic Acid bacteria
  - *Salmonella*
  - *Campylobacter*

doi.org/10.1093/ps/81.6.780

Results: Crop bacteria $\log_{10}$cfu

Treatment	Lactic Acid	*Salmonella*	*Campy*
Control	6.8	1.4	7.3
4% sucrose	7.0	0.0	4.2
4% glucose	7.2	0.3	7.0

doi.org/10.1093/ps/81.6.780

Results: Crop pH & **% Positive**

Treatment	pH	*Salmonella*	*Campy*
Control	6.73	100%	100%
4% sucrose	6.38	100%	40%*
4% glucose	6.40	100%	100%

doi.org/10.1093/ps/81.6.780

Conclusion

- Providing carbohydrate based cocktail lowered Crop pH and level of *Salmonella & Campylobacter*
- No impact on ceca pH, *Salmonella* level, or prevalence
- No impact when cocktail removed for 4 hours to simulate minimum time for catching and transport to the plant

Consensus

- Providing organic acids / cocktail are NOT effective for decontamination of *Salmonella & Campylobacter*

- Providing organic acids / cocktail may help reduce potential for *Salmonella* colonization in chicks

Challenge

- Chicks consume relatively small amounts of feed and water
- Need to providing organic acids at concentrations to be effective but will not depress consumption of feed and/or water on a weekly basis

Summary - *Salmonella*

- Most interventions work in the lab
- Many interventions work with low challenge on the farm
- Some interventions work with high challenge on the farm for short times
- No interventions work thru a 12-hour feed withdrawal 100% at the plant

Questions?