Lysozyme as an alternative to antibiotics in nursery swine diets.

William Oliver

USDA, Agricultural Research Service, Meat Animal Research Center
Clay Center, NE, USA

8/16/2016

Outline

- Antibiotic Use
- FDA Guidance
- Alternatives to antibiotics
- Lysozyme background
  - What is it?
  - Why did we become interested?
- Our work
  - Pilot study
  - Nursery study
  - Immune Challenge Study

Antibiotic Use

- Subtherapeutic levels of antibiotics in feed have been used more than 60 years.
  - Increase growth.
  - Increase feed efficiency.
  - Decrease bacterial infection.

- Increased *Campylobacter* shedding is associated with reduced performance (Wells et al., 2010).

Antibiotic Use

- Feeding antibiotics increases profitability of swine production.
- Pressure to eliminate subtherapeutic antibiotic use throughout the production cycle.
- Safe and effective alternatives to traditional antibiotics will allow swine producers to maintain current levels of efficiency.

Pressure to Decrease Antibiotic Use

PIG PROGRESS

Search...

Breeding Growing / Finishing Pork & Processing Health / Diseases Regi

Outbreaks Health Research

meatingplace

MPBS INDUSTRIES

ETL

MPBS Vacu

HOME NEWS ARCHIVE RESOURCES CLASSIFIED SUBSCRIBE ADVERTISING

SAFETY ZONE BY JAMES MARSDEN

James Marsden is Kansas State University Regent's Distinguished Prof. of Food Safety.

FDA takes on antibiotics in food animals and antibacterial soaps

Facebook Tweet LinkedIn Email ShareThis

Comment Email Blog Print

the use of antibiotics by 10 percent compared to the level in 2009.

"Denmark is a pioneer in working with antibiotic reduction. Other countries envy us the results we have achieved in co-operation with farmers, veterinarians and public authorities," says Danish Minister for Food, Agriculture and Fisheries, Dan Jørgensen.
The reduction means the risk of developing further antimicrobial resistance has been reduced. Dan Jørgensen is very pleased with the results and expresses the

FOOD POLITICS

meatingplace

[redacted]

HOME NEWS ARCHIVE RESOURCES CLASSIFIED SUBSCRIBE ADVERTISING

INDUSTRY NEWS - PM

Two bills curbing antibiotic use on deck in Calif.

Facebook Tweet LinkedIn Email ShareThis

Comment email editors Print

By Chris Scott on 1/21/2014

- Voluntarily notify the FDA of their intent to sign on to these strategies within the next three months.
- Voluntarily put the new guidance into effect within 3 years.
- Agree to a proposed rule to require a veterinarian's prescription to use antibiotics that are presently sold over the counter (the proposal is open for public comment for 90 days at www.regulations.gov. Docket FDA-2010-N-0155).

FDA guidance No. 209 and 213

- Guidance 209 - establishes two voluntary principles
  - The use of medically important antimicrobial drugs in food-producing animals should be limited to uses that are considered necessary for assuring animal health.
  - The use of medically important antimicrobial drugs in food-producing animals should include veterinary oversight or consultation.
- Guidance 213 - outlines the process
  - How a company can withdraw growth claims from the label of products containing medically-important antibiotics.
  - It also describes how a company can apply for a prevention claim, or therapeutic claim, on those same compounds.

FDA Veterinary Feed Directive (VFD)

- Mechanism FDA uses to apply veterinary oversight to a broad range of products used in animal feed.
  - Created in the late 1990s, but was applied to only a small number of products.
- The VFD is the mechanism requiring a producer to get approval from a veterinarian for antibiotics used in animal feed.
  - FDA intends to move all medically-important antibiotics out of over-the-counter status to VFD status.

FDA guidance No. 209 and 213

- List of compounds
  - It is very long
  - Chlortetracycline is on it.
  - Carbadox is not.

Alternatives to Antibiotics

- Antimicrobial peptides
- Antibacterial vaccines
- Bacteriophages
- Probiotics
- Prebiotics
- Bacterial Fermentation Products
- Clay minerals
- Biofilm inhibitors
- Recombinant enzymes
- Rare earth elements
- Plant extracts
- Endolysins
- Egg yolk antibodies
- Essential Oils
- Lysozyme
- Many others

Lysozyme Background

- Naturally occurring enzyme found in bodily secretions (Fleming, 1922)

- 1,4-β-N-acetylmuramidase that enzymatically cleave glycosidic linkage in peptidoglycan component of bacterial cell walls.
  - Hydrolysis products increase IgA secretion, macrophage activation, and clearance of bacterial pathogens.

Lysozyme Background

- Well conserved and a part of innate immune system in most mammals.
- Human breast milk
  - Very low concentrations in sow milk
- Effective against Gram-positive bacteria
  - *In vitro*

Lysozyme Background

- Uses in the food industry
  - Wine making
  - Cheese making
  - Preservative
  - Controlling meat spoilage

Lysozyme Background – Effects on Pigs

- Transgenic sources – human lysozyme
  - Milk of pigs, mice, and goats
  - Rice
- Human medicine

Lysozyme Background – Effects on Pigs

- Growth performance
  - No improvement due to lysozyme
    - Likely due to study design
  - May improve feed efficiency (transgenic rice)

- Gastrointestinal Morphology
  - Variable changes due to lysozyme
  - No changes in jejunum and ileum
  - Slight improvements to duodenal villi height and lamina propria thickness

Lysozyme Background – Effects on Pigs

- Metabolomics
  - 18 known metabolites were changed in serum due to lysozyme consumption (Brundige et al., 2010)
    - Most were "positive"
  - Four indicate increased protein synthesis and growth.
    - methionine, threonine, hydroxyproline were increased
    - Urea was decreased

Lysozyme Background – Effects on Pigs

- Microbial Ecology
  - Reduces total coliforms and *E. coli* (Maga et al., 2006)
  - Microbiome (Maga et al., 2012)
    - Decrease in bacteria associated with disease
      - *Mycobacteriaceae*, *Streptococcaceae*, and *Campylobacterales*
    - Increase in bacteria associated with GI health
      - *Bifidobacteriaceae* and *Lactobacillaceae*

Lysozyme Background – Effects on Pigs

- Lysozyme in milk diets fed to young pigs
  - Improved growth rate and intestinal health

Granulated lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology of 10-day-old pigs[1]

K. D. May,* J. E. Wells,† C. V. Maxwell,* and W. T. Oliver†[2]

*University of Arkansas, Fayetteville 72701; and †USDA, ARS, US Meat Animal Research Center, Clay Center, NE 68933-0166

J. Anim. Sci. 2012. 90:1118–1125
http://dx.doi.org/10.2527/jas.2011-4297

Lysozyme in dry nursery pig diets.

- Hypotheses:
  - Lysozyme improves growth performance in nursery pigs, at least in part, due to improvements in gastrointestinal health.
- Objectives:
  - Determine if lysozyme in nursery diets improves growth performance and gut morphology of pigs weaned from the sow at 24 d of age.

Lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology in nursery pigs[1]

W. T. Oliver[2] and J. E. Wells

USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933-0166

J. Anim. Sci. 2013.91:3129–3136
doi:10.2527/jas2012-5782

Experimental Design

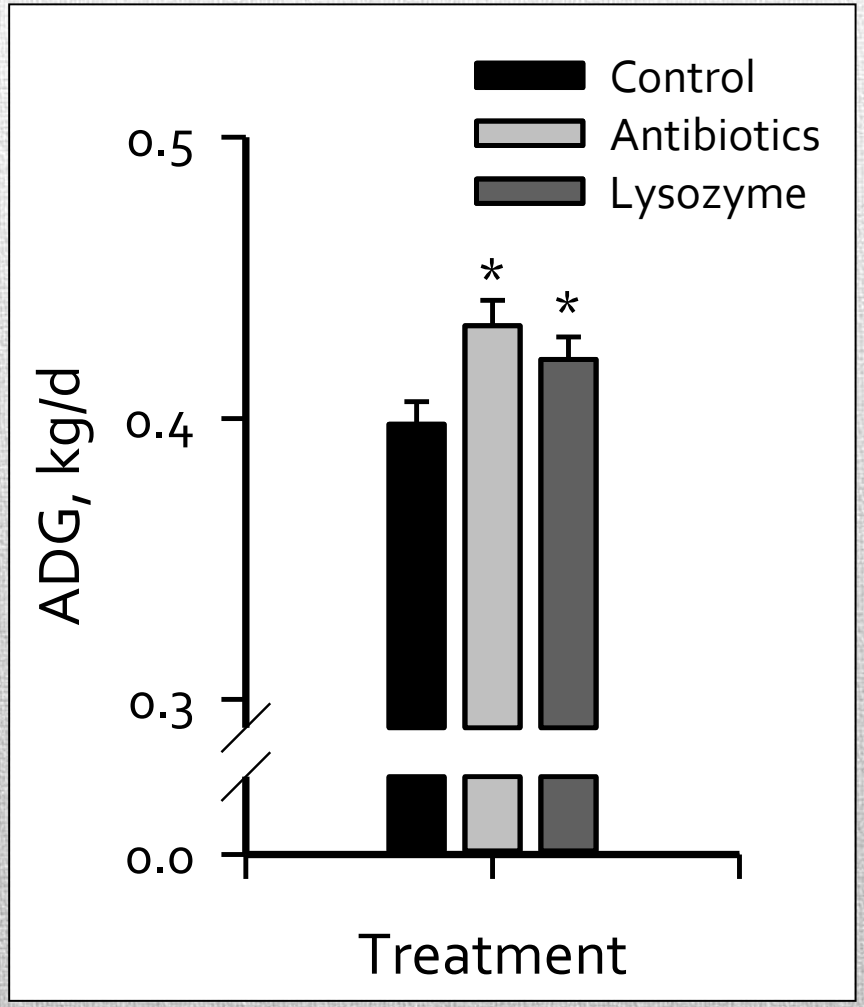
- Two replicates of 96 pigs(N=192; mixed gender)
  - Removed from the sow at 24 days of age.
  - Blocked by litter and gender and assigned to one of 12 pens (24 for the entire study; n=8).
  - Blocks randomly assigned to an experimental diet and fed for 28 days (two phases).
    - Control: Typical nursery diets
    - Control + Antibiotics: Carbadox (55 mg/kg) and $CuSO_4$ (250mg/kg)
    - Control + Lysozyme: 100 mg/kg (Entegard, Bioseutica, Inc.)

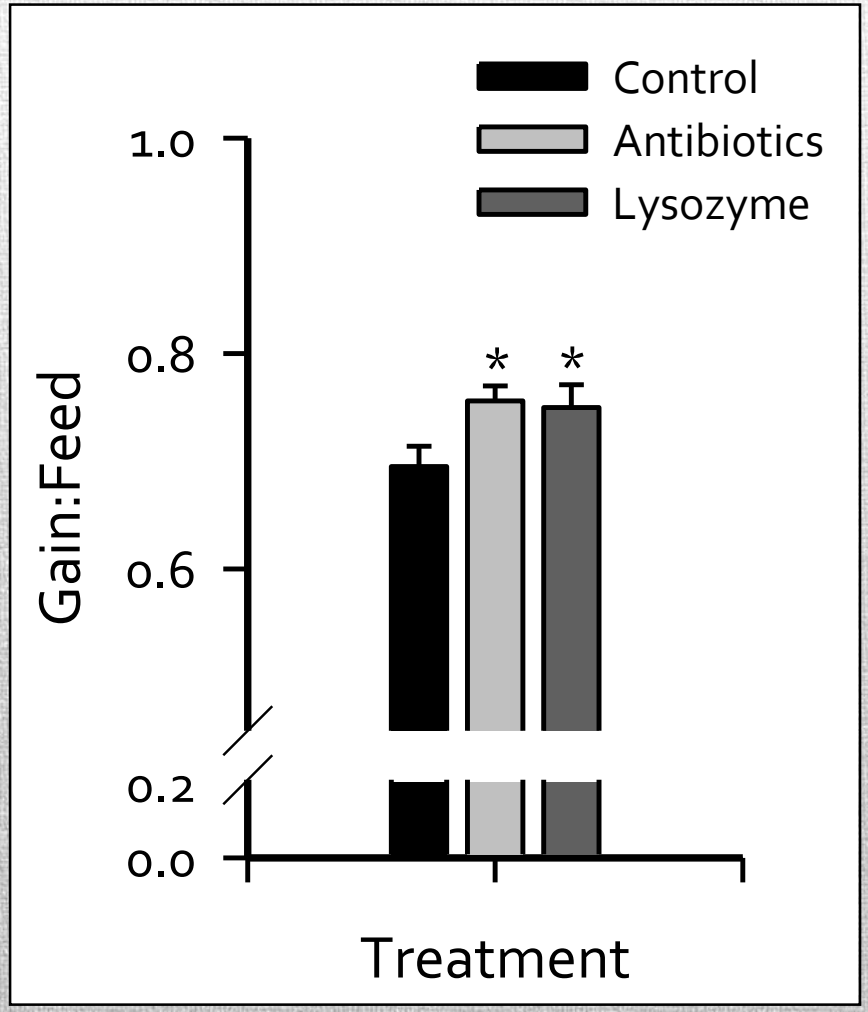
Diets

	Phase 1 (d 0 to 14)			Phase 2 (d 14 to 28)		
Ingredients, %	Control	C + A	C + Lyso	Control	C + A	C + Lyso
Corn	50.8	49.6	50.5	63.3	62.1	63.0
Soybean meal, 465 g/kg	24.3	24.4	24.3	26.5	26.6	26.6
Fish Meal	5.0	5.0	5.0	2.5	2.5	2.5
Blood meal	1.3	1.3	1.3	1.3	1.3	1.3
Whey	12.5	12.5	12.5	0.0	0.0	0.0
Soybean oil	3.0	3.0	3.0	3.0	3.0	3.0
Carbadox	0.0	1.0	0.0	0.0	1.0	0.0
Copper sulfate	0.0	0.1	0.0	0.0	0.1	0.0
Lysozyme	0.0	0.0	0.3	0.0	0.0	0.3

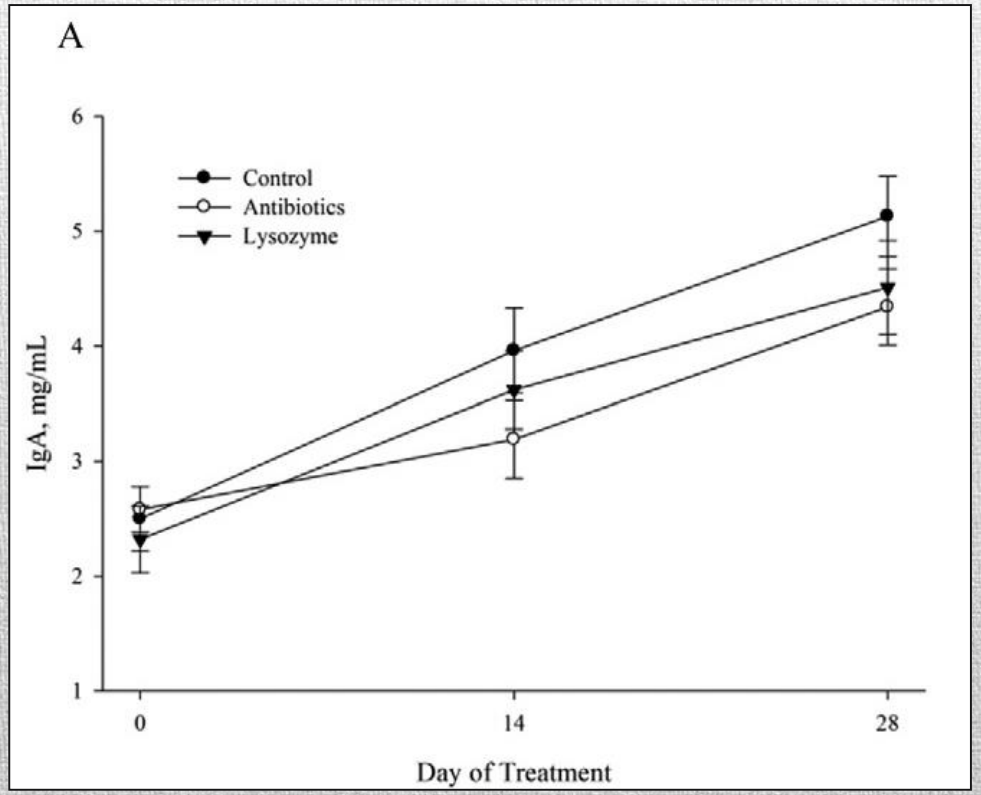
*Plus vitamins minerals, and purified crystalline amino acids

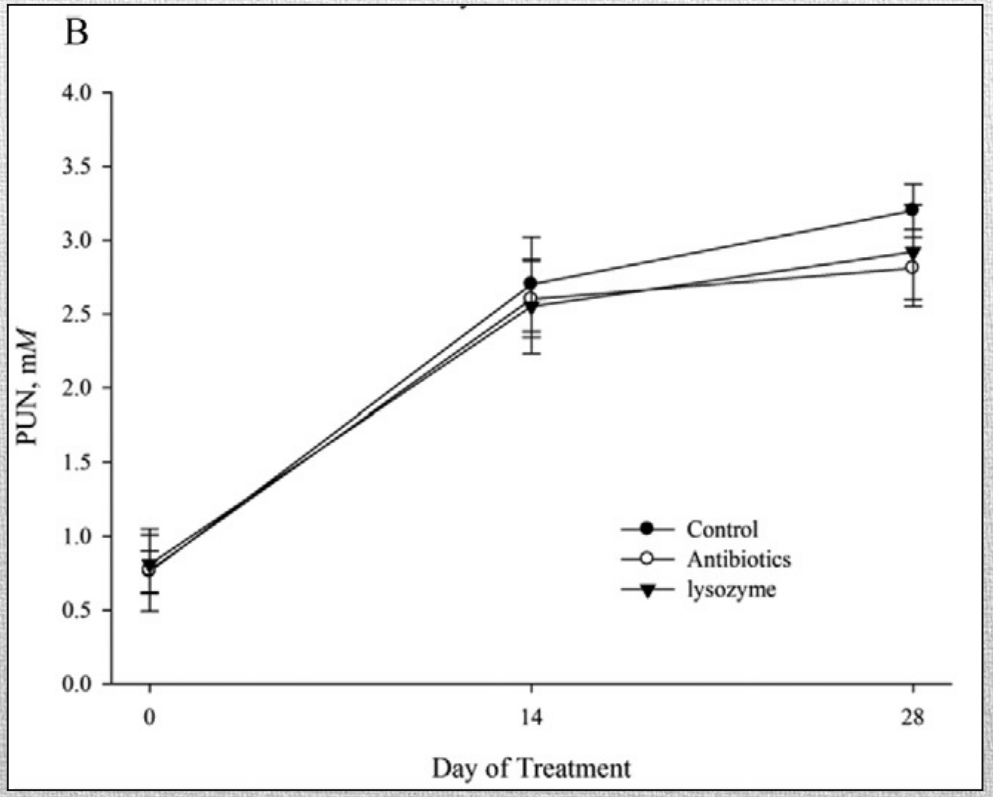
ADG and Gain:Feed





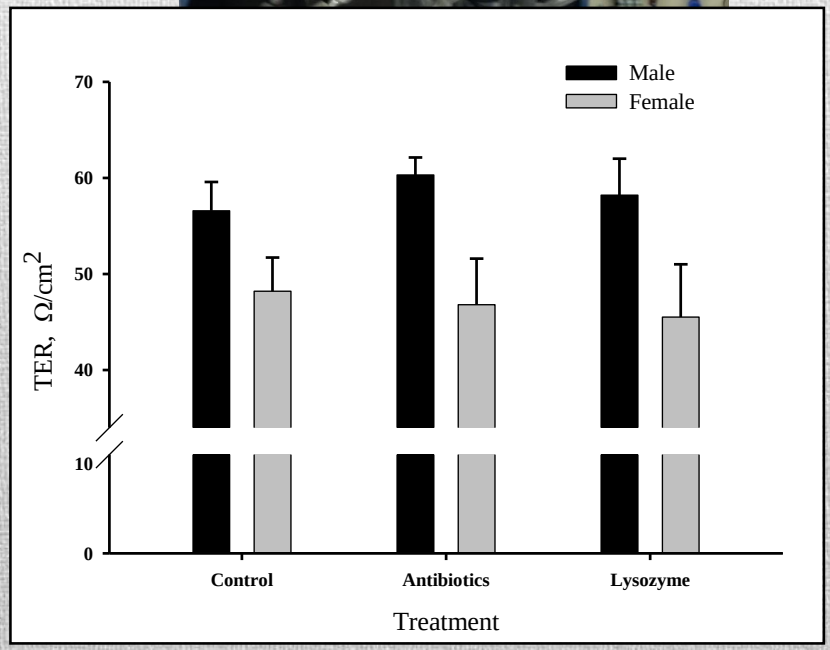
Circulating IGA and PUN



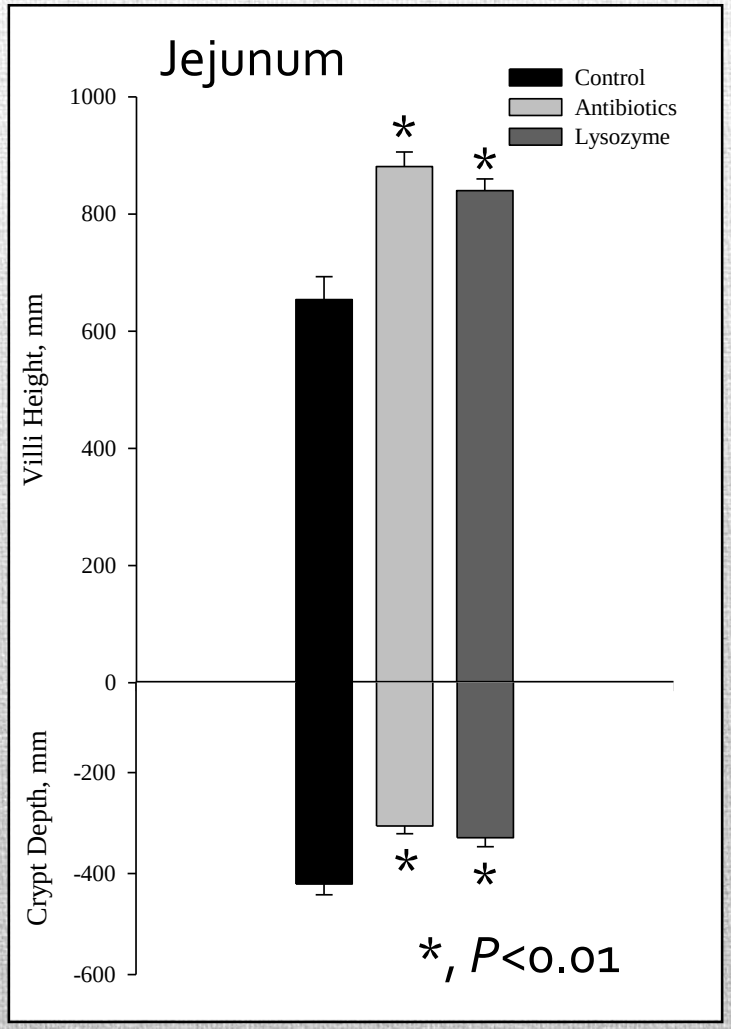


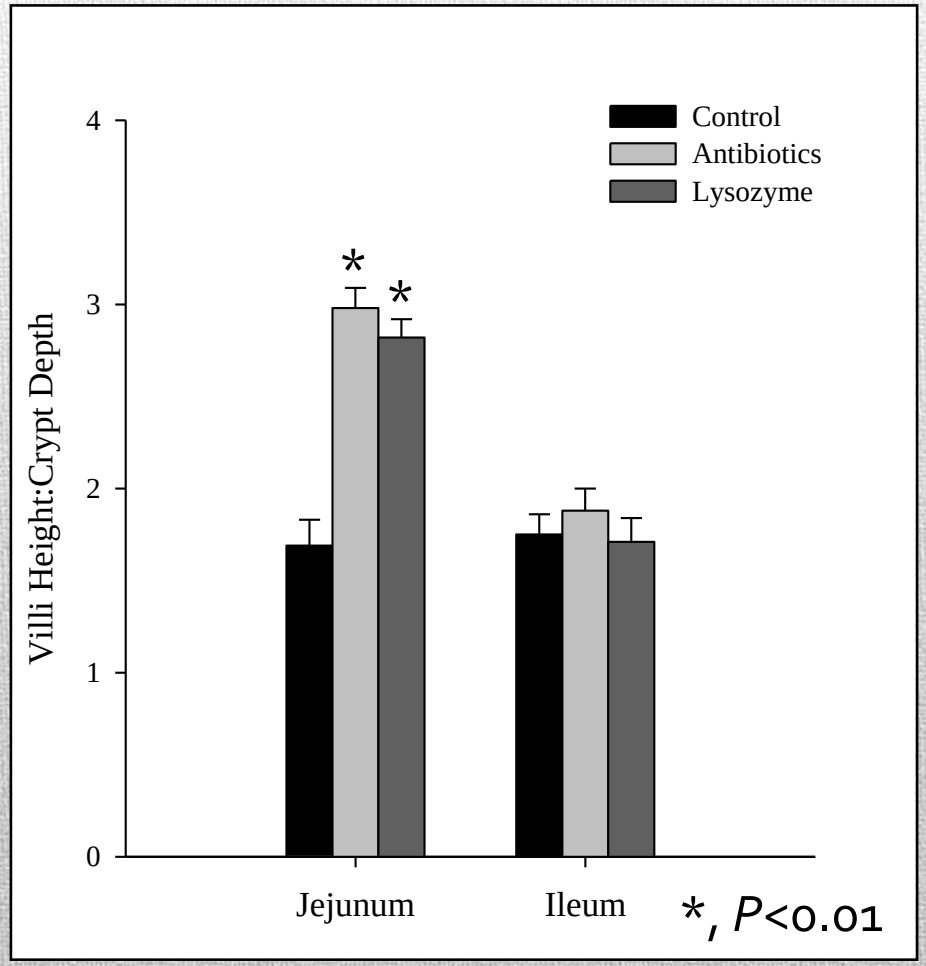
Transepithelial Electrical Resistance

- Lysozyme and antibiotics do not appear to affect barrier function in nursery pigs.



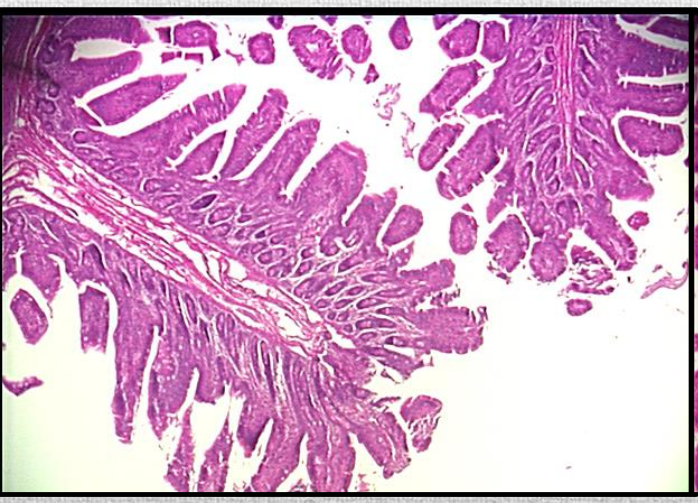
Gut Morphology



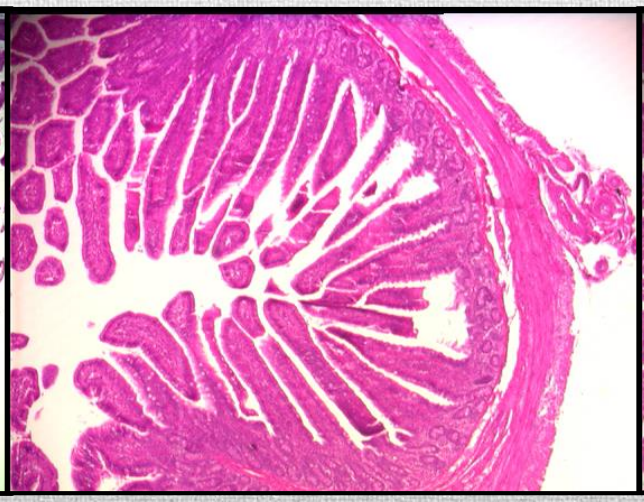


Gut Morphology

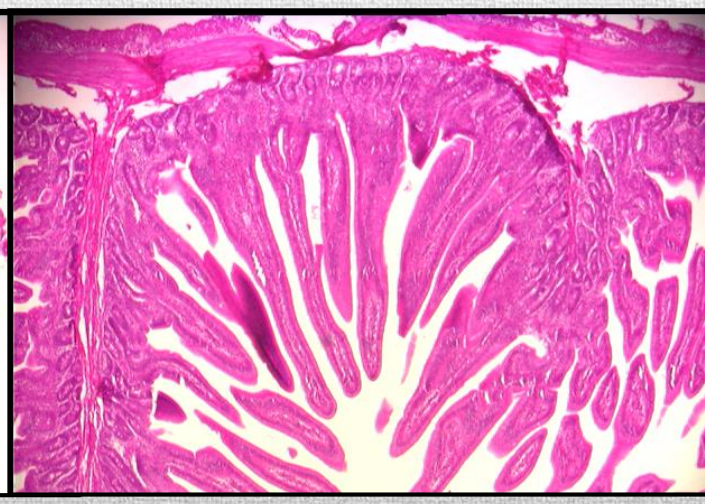
CONTROL



CONTROL + ANTIBIOTIC



CONTROL + LYSOZYME



Summary

- In nursery pigs:
  - Lysozyme increases growth, similarly to antibiotics.
  - Lysozyme increases feed efficiency, similarly to antibiotics.
  - It appears that the improvements in performance are due to, at least in part, improved GIT health.
    - Likely due to improved absorption.

Conclusion

- Lysozyme is a suitable alternative to carbadox/copper sulfate diets fed to pigs weaned from the sow at 24 d of age.

Lysozyme – More Questions?

- We think that lysozyme decreases pathogen shedding.
  - Does it improve the health of the pig?

- We know that the activated immune system, even chronic, low activation, is detrimental to pig performance.
  - Can lysozyme or antibiotics improve growth during an immune stimulation?

Lysozyme – Immune Challenge

- Effect of lysozyme or antibiotics in ameliorating the effects of an indirect disease challenge.
  - Funded by NPB

Lysozyme as an alternative to antibiotics improves performance in nursery pigs during an indirect immune challenge[1,2]

W. T. Oliver*[3], J. E. Wells*, and C. V. Maxwell†

*USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933-0166; and †University of Arkansas, Fayetteville 72701

J. Anim. Sci. 2014.92:4927–4934
doi:10.2527/jas2014-8033

Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Effect of lysozyme or antibiotics on faecal zoonotic pathogens in nursery pigs

J.E. Wells, E.D. Berry, N. Kalchayanand, L.A. Rempel, M. Kim* and W.T. Oliver

USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA

Lysozyme – Immune Challenge

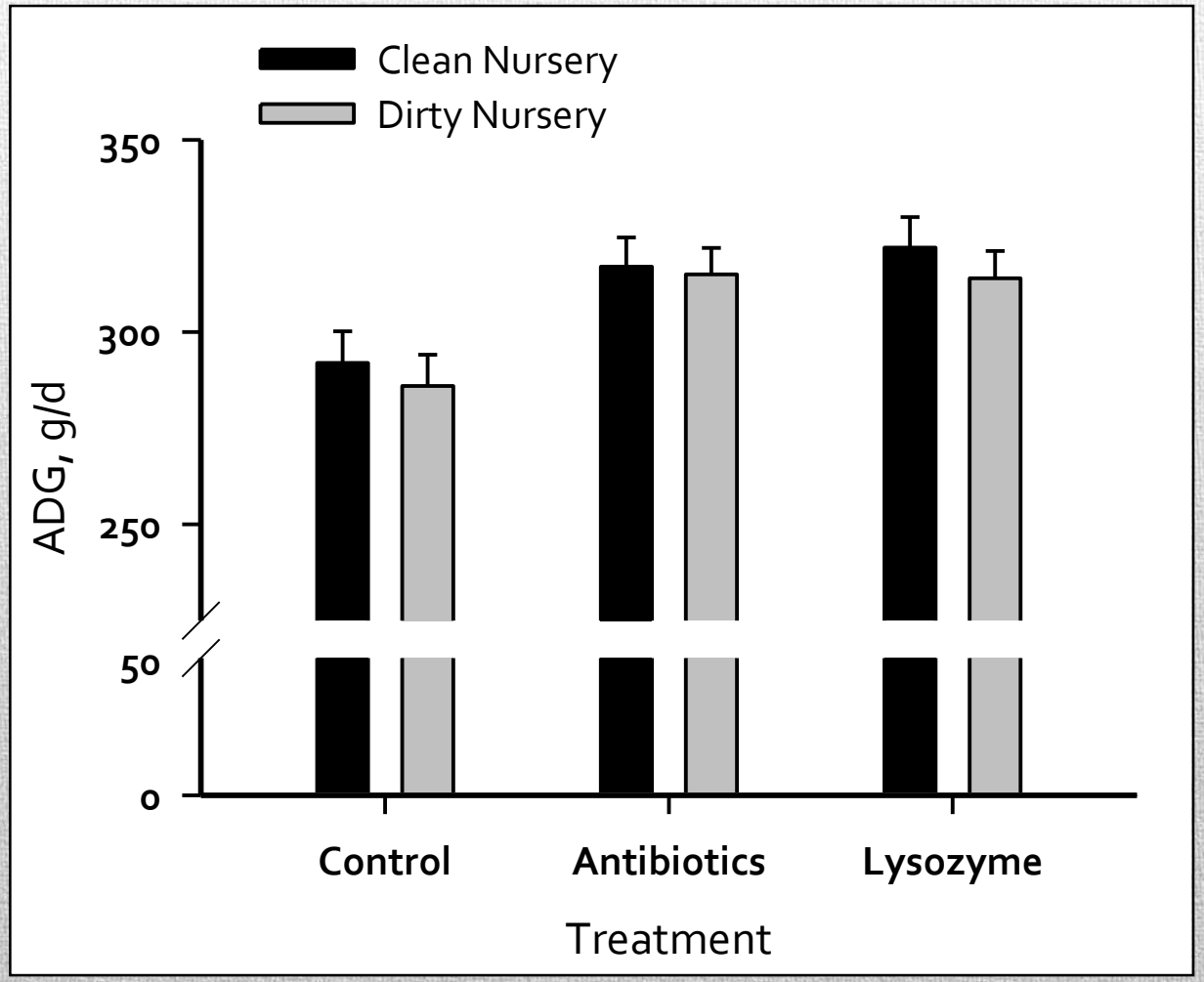
- Clean/dirty nursery model; Pigs reared in either a fully cleaned and disinfected nursery, or one that was left untouched since the last group of pigs

- Pigs reared in a dirty nursery:
  - Usually have shown slower growth performance
  - Always have increased immune response
    - Altered lymphocyte (TCR/CD4/CD8) subset phenotypes
    - Cytokine response
    - Acute phase protein response

Experimental Design

- Two replicates of 600 pigs(N=1,200; mixed gender)
  - Removed from the sow at 26 days of age and weaned into either a clean or dirty nursery
    - Split plot design
  - Blocked by litter and gender and assigned to one of 36 pens per room (72 for the entire study; n=12).
  - Blocks assigned to an experimental diet and fed for 28 days (two phases).
    - Control: Typical nursery diets
    - Control + Antibiotics: Chlortetracycline (55 mg/kg) and Denegard (1.65 g/kg).
    - Control + Lysozyme: 100 mg/kg (Entegard, Bioseutica, Inc.)

Performance



- d 0-28
  - Diet, $P<0.01$
  - Nursery, $P=0.11$

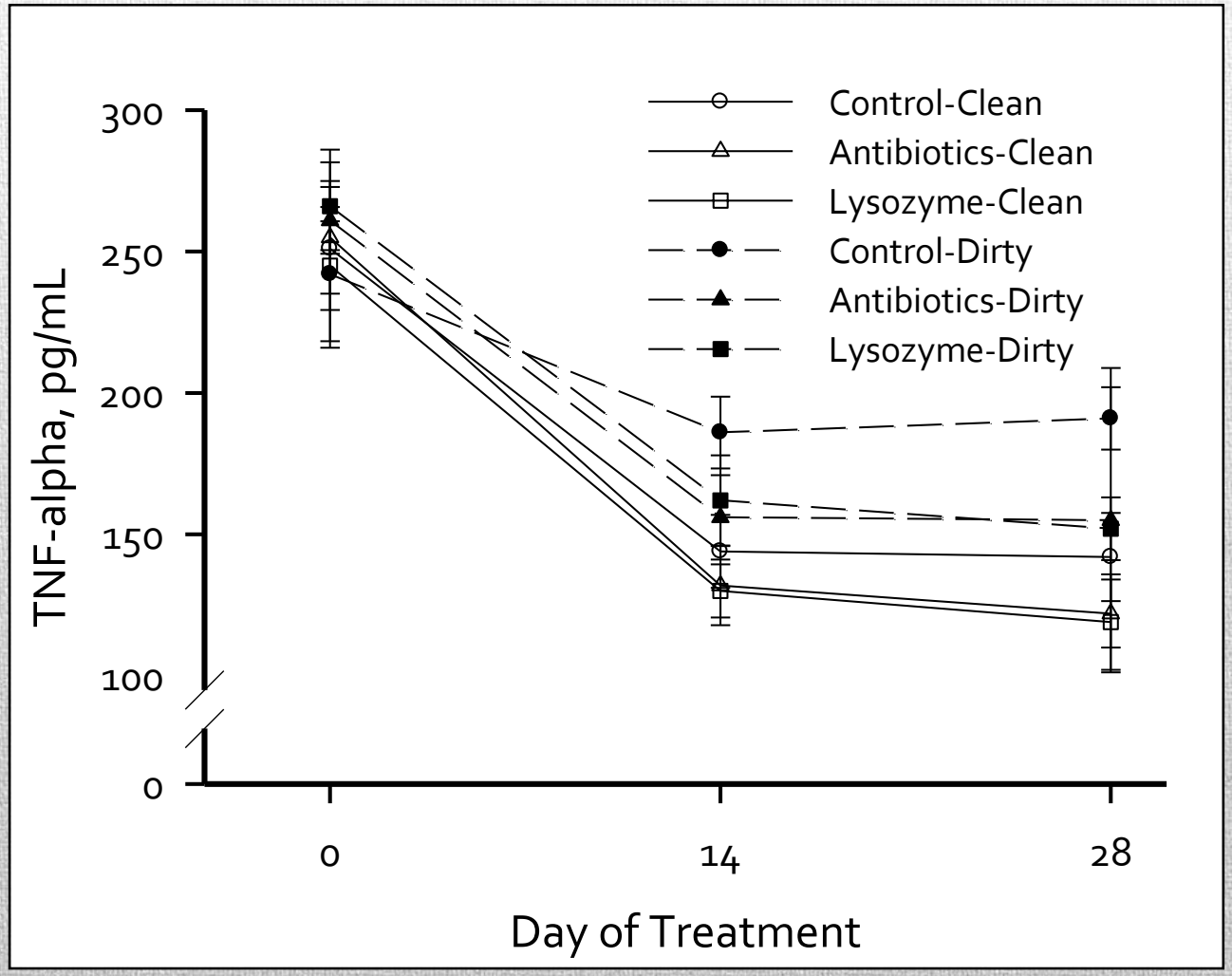
Performance

	Clean Nursery			Dirty Nu						
	Control	C + A	C + Lyso	Control	C + A					
ADFI, kg·pen^{-1}·d^{-1}										
d 0 to 28								0.112	0.866	0.870
G:F										
d 0 to 28								0.005	0.049	0.389
Days to Market	96.4	92.1	92.8	98.1	93.6	93.4	0.3	0.041	0.086	0.682

The increase in G:F was due entirely to improvements during the first two weeks of study.

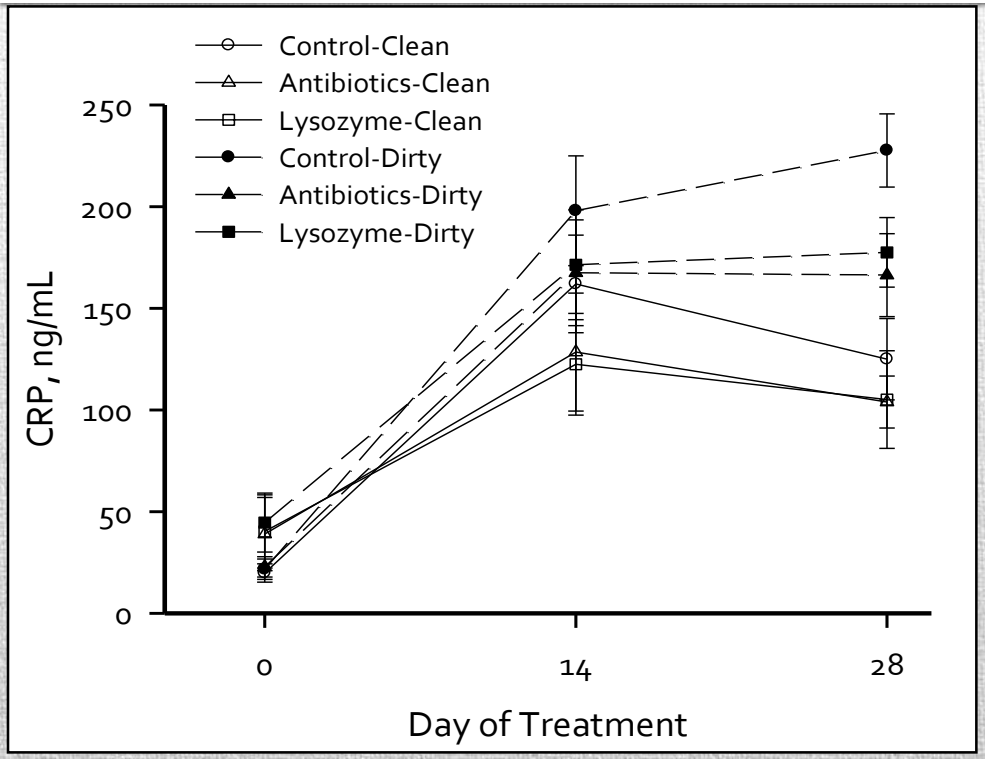
Both lysozyme and antibiotics improved days to market by about 5 d.

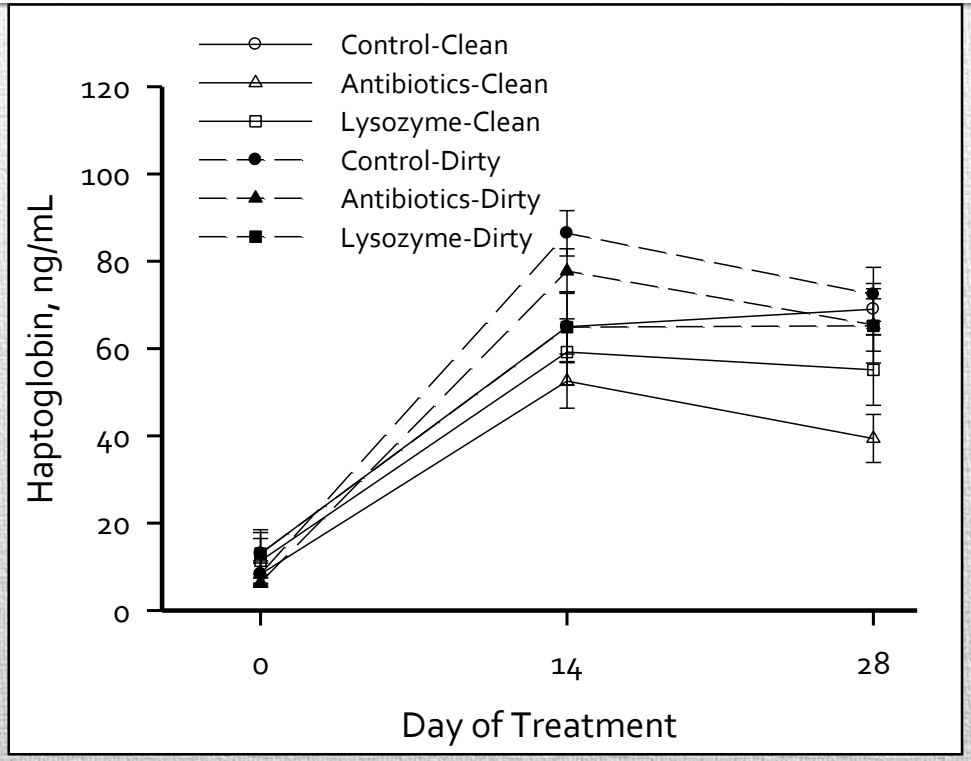
Cytokines



- Diet, $P<0.04$
- Diet*Nursery, $P<0.01$

Acute Phase Proteins





- Diet, $P<0.01$
- Nursery, $P<0.01$

- Diet, $P<0.02$
- Nursery, $P<0.09$

Accretion Rates

	Accretion rates, g/d			
Clean Nursery	Protein	Lipid	Ash	Water
C, d 0 to28	45.3 ± 1.2	19.4 ± 1.9	11.9 ±	
C + A, d 0 to 28	51.0 ± 1.2	16.9 ± 1.8	12.1 ±	
C + Lyso, d 0 to 28	47.7 ± 1.0	16.2 ± 1.4	10.9 ±	
Dirty Nursery				
C				4.5 ± 6.2
				6.6 ± 5.5
				2.5 ± 6.0

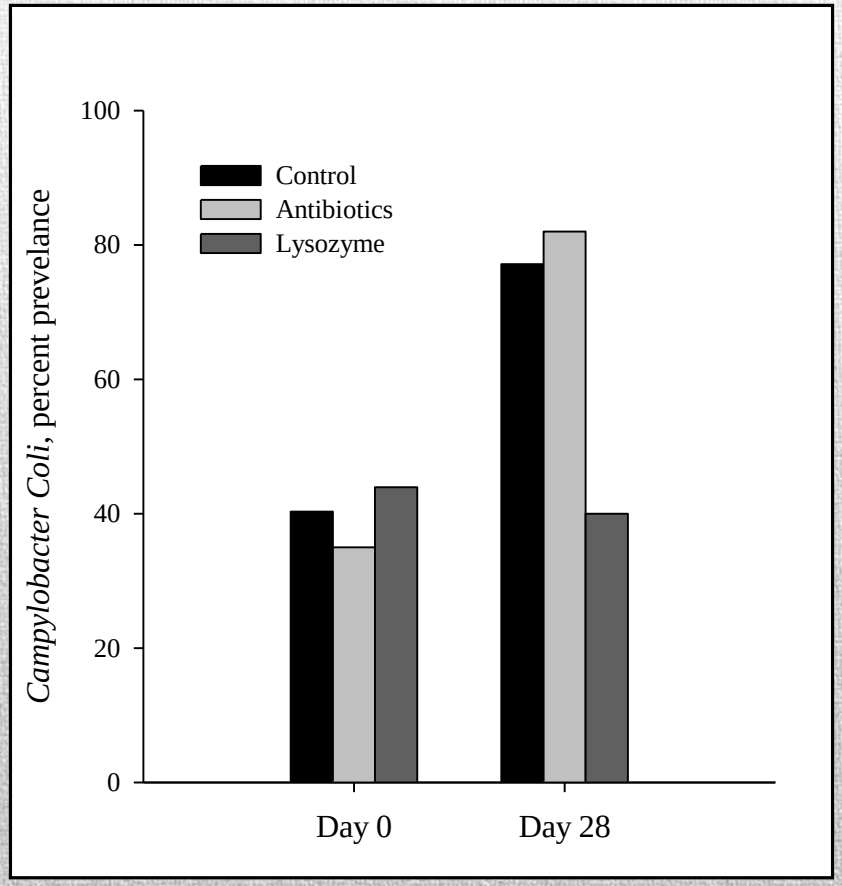
Lysozyme and antibiotics decreased lipid accretion by 12-16%.

Lysozyme and antibiotics tended to increase protein accretion by 5-10%.

- Nursery, NS

Pathogen Shedding

- Shedding of bacteria potentially harmful to humans.
  - *Salmonella*
  - Shigatoxigenic *E. coli*.
  - EHEC virulence genes
  - *Campylobacter coli*
- Unlike CTC/Denegard, lysozyme eliminates the normal increase in *Campylobacter* shedding in the nursery.



Summary and Conclusion

- Pigs in the dirty nursery had performance and cytokine and APP levels indicative of a chronic immune challenge.
- Pig consuming lysozyme or antibiotics had improved growth performance, regardless of immune status.
- Pigs consuming lysozyme or antibiotics had higher protein and lower lipid accumulation, regardless of immune status

- Thus, lysozyme is a suitable alternative to antibiotic in nursery diets, including during a chronic immune stimulation.

Lysozyme is a suitable alternative to antibiotics in nursery diets.

- Pig consuming lysozyme or antibiotics had improved growth performance, regardless of immune status.
- Pigs consuming lysozyme or antibiotics had higher protein and lower lipid accumulation, regardless of immune status
- Lysozyme in the diet can reduce fecal shedding of *Campylobacter* spp. from nursery swine.
- Improvements in performance are, at least party, due to improved gastrointestinal health.

Oliver and Wells *Journal of Animal Science and Biotechnology* (2015) 6:35
DOI 10.1186/s40104-015-0034-z

JOURNAL OF ANIMAL SCIENCE AND BIOTECHNOLOGY

REVIEW Open Access

CrossMark

Lysozyme as an alternative to growth promoting antibiotics in swine production

W. T. Oliver* and J. E. Wells

Acknowledgments

- USMARC
  - Jim Wells
  - Dee Kucera
  - Shanda Watts
  - Bruce Larsen
  - Nor Kalchayanand
  - Bruce Jasch
  - Elaine Berry
  - Shannon Ostdiek
  - Donna Griess
  - Jessica Rohan
  - Pat Nuss
  - Purchasing
- Swine Operations
  - Devin Gandy
  - Ron Pooschke
  - Jenell Wood
  - Deb Loudon
  - Garrett Nathan
  - Wayne Peshek
  - Michelle Anderson
- University of Arkansas
  - Charlie Maxwell
  - Kim May
- NEMRU
  - Kathy Rohren
  - Harvey Freetly
  - Chris Haussler
  - Amanda Lindholm-Perry
  - Linda Flathman
  - Kristin Hales
  - Cindy Felber
- National Pork Board

Questions?