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
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).
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

FDA takes on antibiotics in food animals and antibacterial soaps

FDA takes on antibiotics in food animals and antibacterial soaps

Two bills curbing antibiotic use on deck in Calif.

Two bills curbing antibiotic use on deck in Calif.

INDUSTRY NEWS - PM

Two bills curbing antibiotic use on deck in Calif.

Two bills curbing antibiotic use on deck in Calif.

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.
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.
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
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
**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

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

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

©2012 American Society of Animal Science. All rights reserved.

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.
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 CuSO4 (250mg/kg)
  - Control + Lysozyme: 100 mg/kg (Entegard, Bioseutica, Inc.)
<table>
<thead>
<tr>
<th>Ingredients, %</th>
<th>Control</th>
<th>C + A</th>
<th>C + Lyso</th>
<th>Control</th>
<th>C + A</th>
<th>C + Lyso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>50.8</td>
<td>49.6</td>
<td>50.5</td>
<td>63.3</td>
<td>62.1</td>
<td>63.0</td>
</tr>
<tr>
<td>Soybean meal, 465 g/kg</td>
<td>24.3</td>
<td>24.4</td>
<td>24.3</td>
<td>26.5</td>
<td>26.6</td>
<td>26.6</td>
</tr>
<tr>
<td>Fish Meal</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Blood meal</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Whey</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Carbadox</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0.0</td>
<td>0.0</td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Plus vitamins, minerals, and purified crystalline amino acids.
ADG and Gain:Feed
Circulating IGA and PUN

(A) IgA, ng/mL
(B) PUN, mM
Transepithelial Electrical Resistance

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

**Jejunum**

- Villi Height: Crypt Depth
- Control, Antibiotics, Lysozyme

* *, $P<0.01$

**Ileum**

- Villi Height: Crypt Depth
- Control, Antibiotics, Lysozyme

* *, $P<0.01$
Gut Morphology

CONTROL

CONTROL + ANTIBIOTIC

CONTROL + LYSOZYME
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.
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?
Effect of lysozyme or antibiotics in ameliorating the effects of an indirect disease challenge.

- Funded by NPB
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, $P=0.11$
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

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Lipid</th>
<th>Ash</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clean Nursery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>45.3 ± 1.2</td>
<td>19.4 ± 1.9</td>
<td>11.9 ± 0.7</td>
<td>229.2 ± 6.7</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>51.0 ± 1.2</td>
<td>16.9 ± 1.8</td>
<td>12.1 ± 0.4</td>
<td>239.8 ± 7.9</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>47.7 ± 1.0</td>
<td>16.2 ± 1.4</td>
<td>10.9 ± 0.6</td>
<td>235.5 ± 4.4</td>
</tr>
<tr>
<td><strong>Dirty Nursery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>44.9 ± 1.3</td>
<td>18.9 ± 2.0</td>
<td>11.6 ± 0.6</td>
<td>224.5 ± 6.2</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>50.6 ± 1.1</td>
<td>15.4 ± 1.8</td>
<td>11.8 ± 0.6</td>
<td>236.6 ± 5.5</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>48.1 ± 1.5</td>
<td>15.0 ± 1.6</td>
<td>10.8 ± 0.8</td>
<td>232.5 ± 6.0</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clean Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>45.3 ± 1.2</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>51.0 ± 1.2</td>
<td>16.9 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>47.7 ± 1.0</td>
<td>16.2 ± 1.4</td>
</tr>
<tr>
<td><strong>Dirty Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>44.9 ± 1.3</td>
<td>18.9 ± 2.0</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>50.6 ± 1.1</td>
<td>15.4 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>48.1 ± 1.5</td>
<td>15.0 ± 1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clean Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>45.3 ± 1.2</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>51.0 ± 1.2</td>
<td>16.9 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>47.7 ± 1.0</td>
<td>16.2 ± 1.4</td>
</tr>
<tr>
<td><strong>Dirty Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>44.9 ± 1.3</td>
<td>18.9 ± 2.0</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>50.6 ± 1.1</td>
<td>15.4 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>48.1 ± 1.5</td>
<td>15.0 ± 1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clean Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>45.3 ± 1.2</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>51.0 ± 1.2</td>
<td>16.9 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>47.7 ± 1.0</td>
<td>16.2 ± 1.4</td>
</tr>
<tr>
<td><strong>Dirty Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>44.9 ± 1.3</td>
<td>18.9 ± 2.0</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>50.6 ± 1.1</td>
<td>15.4 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>48.1 ± 1.5</td>
<td>15.0 ± 1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clean Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>45.3 ± 1.2</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>51.0 ± 1.2</td>
<td>16.9 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>47.7 ± 1.0</td>
<td>16.2 ± 1.4</td>
</tr>
<tr>
<td><strong>Dirty Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>44.9 ± 1.3</td>
<td>18.9 ± 2.0</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>50.6 ± 1.1</td>
<td>15.4 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>48.1 ± 1.5</td>
<td>15.0 ± 1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clean Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>45.3 ± 1.2</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>51.0 ± 1.2</td>
<td>16.9 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>47.7 ± 1.0</td>
<td>16.2 ± 1.4</td>
</tr>
<tr>
<td><strong>Dirty Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>44.9 ± 1.3</td>
<td>18.9 ± 2.0</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>50.6 ± 1.1</td>
<td>15.4 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>48.1 ± 1.5</td>
<td>15.0 ± 1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clean Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>45.3 ± 1.2</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>51.0 ± 1.2</td>
<td>16.9 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>47.7 ± 1.0</td>
<td>16.2 ± 1.4</td>
</tr>
<tr>
<td><strong>Dirty Nursery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C, d 0 to 28</td>
<td>44.9 ± 1.3</td>
<td>18.9 ± 2.0</td>
</tr>
<tr>
<td>C + A, d 0 to 28</td>
<td>50.6 ± 1.1</td>
<td>15.4 ± 1.8</td>
</tr>
<tr>
<td>C + Lyso, d 0 to 28</td>
<td>48.1 ± 1.5</td>
<td>15.0 ± 1.6</td>
</tr>
</tbody>
</table>
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.
Pigs in the dirty nursery had performance and cytokine and APP levels indicative of a chronic immune challenge.

Pigs 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 partly, due to improved gastrointestinal health.
## 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?