Meta-analysis of Broiler Research Shows that Varium Results in Feed Efficiency Equal to Antibiotics

R. L. Cravens, F. Chi*, S. Ching, S. L. Johnston
Amlan International, Chicago, USA
ATA Conference, Paris, December, 14, 2016
<table>
<thead>
<tr>
<th>Discussion</th>
<th>Time</th>
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<tbody>
<tr>
<td>Necrotic Enteritis, Bacterial Toxins and Intestinal Health</td>
<td>15 min</td>
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<tr>
<td>Varium Meta-analysis Methods</td>
<td>10 min</td>
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<tr>
<td>Wrap up and Questions</td>
<td>5 min</td>
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</table>
Necrotic Enteritis

• Over 2 Billion US dollars is estimated to be the annual cost of necrotic enteritis (NE) in broiler production worldwide.

• Clostridium perfringens pathogen (CPP), a gram positive and anaerobic bacteria, plays key role in NE development.

• CPP toxins, such as $\alpha$-toxin, NE B-like (NetB) and $\beta_2$-toxin etc. are shown to cause lesions in the intestine.
Clostridium perfringens Toxins

<table>
<thead>
<tr>
<th>Strains</th>
<th>Toxins</th>
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<tbody>
<tr>
<td>A</td>
<td>$\alpha$</td>
</tr>
<tr>
<td>B</td>
<td>$\beta$, $\beta_2$, $\tau$</td>
</tr>
<tr>
<td>C</td>
<td>$\alpha$, $\beta$</td>
</tr>
<tr>
<td>D</td>
<td>$\alpha$, $\varepsilon$</td>
</tr>
<tr>
<td>E</td>
<td>$\alpha$, $\iota$</td>
</tr>
</tbody>
</table>

- $\alpha$-toxin vs NetB toxin

- 14 novel toxins had been identified (33 – 54 KD), including enterotoxin, NetB, perfringolysin (pore-forming toxin), enzymes (collagenase, protease, hyaluronidase etc.)

- Host (specie) specific? For instance, NetB dominant in poultry.
Sub-Clinical Enteritis

0 - Normal: No NE lesions, intestine has normal elasticity (roll back on itself after being opened)
NE Lesion Score 1, 2

1 - Mild: Thin and flaccid intestinal wall (intestine remains flat when opened and does not roll back into normal position); thickened mucus covering mucus membrane
2 - Moderate: Noticeable reddening and swelling of the intestinal wall; minor ulceration and necrosis of the intestine membrane; excess mucus
3 - Severe: Extensive area of necrosis and ulceration of the small intestine membrane; significant hemorrhage, layer of fibrin and necrotic debris on the mucus membrane (Turkish towel appearance)

4 - Dead or moribund bird (bird that would likely die within 24 hours) with NE gross lesions scored 2 or more
Clostridium perfringens

- It grows at a temperature between 15°C and 50°C, with an optimum growth at 45°C. The generation time for most strains is less than 20 minutes at 33°C to 49°C.

- Clostridium perfringens is a spore forming pathogen, can tolerate 100°C for two hours.

- Can’t synthesize its own amino acids (auxotrophy, needs >10 AAs); therefore, requires AAs from its environments, such as proteins from feed and mucin in the intestine.
Clostridium perfringens, continue

- Typically, it can be found in the crop, duodenum, jejunum, ileum, and ceca of healthy birds.

- It colonizes the intestines of broiler chickens within a few hours after hatching and the numbers increase gradually after initial colonization. The intestine of healthy birds contains up to $10^5$ colony-forming units per gram of intestinal contents.
Bacterial Imbalance Leads to an Overgrowth of *Clostridium perfringens*

Other Pathogens

- E. Coli
- Salmonella
Bacterial Exotoxins Damage Intestinal Health & Function

Disease Cascade:

• Toxins kill enterocytes

• Breakdown of tight junctions between enterocytes

• Influx of pathogens past epithelial layer enter into circulation “leaky gut”

• Electrolytes secrete into intestine causing diarrhea

• Villus shrink reducing nutrient absorption
To Promote Growth, Without AGPs, Additives Must Affect Multiple Aspects of the Intestinal Environment

1. Reduce challenge from pathogenic bacteria and biotoxins
2. Strengthen the Intestinal Barrier to Defend Against Invasion
3. Activate immune system to prepare against antigens
Bacterial Toxins *in vitro* Binding Tests

USDA, Beltsville, MD
### C. perfringens Toxins

<table>
<thead>
<tr>
<th></th>
<th>α toxin</th>
<th>NetB toxin</th>
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</thead>
<tbody>
<tr>
<td>Amount (µg/ml)</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

USDA, Beltsville MD, In vitro Analysis
E. coli Toxins

USDA, Beltsville MD. In vitro Analysis
Agglutination: Varium and *Salmonella* Bacteria

Source: University of Georgia, Athens, GA, 2016
Agglutination: Varium and *Salmonella* Bacteria

Source: University of Georgia, Athens, GA, 2016
Varium™ Feeding Trials

• 5 research institutes, 9 trials:
  – USDA, Beltsville, Maryland
  – Southern Poultry Research, Athens, Georgia
  – Colorado Quality Research, Wilmington, Colorado
  – Virginia Diversified Research, Harrisonburg, Virginia
  – Life Science Dynamics, Mounds View, Minnesota
Under Necrotic Enteritis Disease Challenge
Mortality (%)
Mortality (%)
Serum α Toxin Levels

USDA, 2013

(μg/ml)

With NE

Virginiamycin

0.85

0.91

0.73

0.25%
Serum NetB Toxin Levels

USDA, 2013

With NE: 0.34
Virginiamycin: 0.32
0.25%
Calcium Montmorillonite-Based Dietary Supplement Attenuates Necrotic Enteritis Induced by *Eimeria maxima* and *Clostridium perfringens* in Broilers†

Hyun S. Lillehoj¹, Sung H. Lee¹*, Soon S. Park¹, Misun Jeong¹, Yeaseul Lim¹, Greg F. Mathis², Brett Lumpkins², Fang Chi³, Chris Ching³ and Ron L. Cravens³

¹Animal Biosciences and Biotechnology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705 USA
²Southern Poultry Research, Inc., Athens, GA 30607 USA
³Amlan International, Chicago, IL 60611 USA
Under Dirty Litter Challenge
Body Weight (g), D42

- Non-medicated Control: 2168
- Medicated Control: 2214
- Phytogenic Product + Fatty Acid: 2168
- Formulated Yeast 1: 2145
- Formulated Yeast 2: 2123
- Formulated Yeast 3: 2136
- Antimicrobial Mineral: 2091

Virgin Diversify Research, 2014
Feed Conversion

- Non-medicated Control: 1.75
- Medicated Control: 1.66
- Phytogenic Product + Fatty Acid: 1.77
- Formulated Yeast 1: 1.76
- Formulated Yeast 2: 1.74
- Formulated Yeast 3: 1.77
- Antimicrobial Mineral: 1.83

0.25%
Under No Disease Challenge
Feed Conversion D28

1.48

Control

1.43

BMD

1.43

B

0.25%

Life Science Dynamics, 2015
Feed Conversion D42

1.73

Control

1.69

BMD

0.25%

Southern Poultry Research, 2014
Meta Analysis of Varium Data
## Meta Analysis

<table>
<thead>
<tr>
<th>Description</th>
<th>Varium – Antibiotic - NE</th>
<th>Weight gain, kg</th>
<th>Feed intake, kg</th>
<th>FCR adjusted</th>
<th>FCR, %</th>
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</thead>
<tbody>
<tr>
<td>No - No -No</td>
<td>1.353 a</td>
<td>2.233</td>
<td>1.665 cd</td>
<td></td>
<td>100.0</td>
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<tr>
<td>No - No -Yes</td>
<td>1.226 d</td>
<td>2.207</td>
<td>2.055 a</td>
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<td>123.4</td>
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<tr>
<td>No - Yes -No</td>
<td>1.330 ab</td>
<td>2.094</td>
<td>1.611 d</td>
<td></td>
<td>96.8</td>
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<tr>
<td>Yes - No -No</td>
<td>1.340 ab</td>
<td>2.129</td>
<td>1.620 cd</td>
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<td>97.3</td>
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<tr>
<td>No - No -Yes</td>
<td>1.275 cd</td>
<td>2.162</td>
<td>1.794 b</td>
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<td>107.7</td>
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<tr>
<td>Yes - No -Yes</td>
<td>1.302 bc</td>
<td>2.205</td>
<td>1.794 b</td>
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<td>107.7</td>
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<tr>
<td>Yes - Yes -Yes</td>
<td>1.285 bcd</td>
<td>2.132</td>
<td>1.718 bc</td>
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<td>103.2</td>
</tr>
</tbody>
</table>

Different letter within a column indicates a significant difference of $p < 0.05$
Relative FCR to the Non-Challenged Control
Growth Performance Proven in Clinical Studies

- Growth equal to AGP with No Challenge
- Growth equal to AGP with Disease Challenge
- Reduced Mortality when used with AGP
- Superior performance vs. 5 alternatives to AGPs
Varium Triple-Action Formula Provides the Most Complete Protection from Loss

① Reduces level of bacterial toxins and pathogenic bacteria

② Provides cellular energy to strengthen enterocytes

③ Primes immune function to prepare against disease
Acknowledgements

• Dr. Lillehoj etc., USDA, Beltsville, MD, USA
• Dr. Lee, University of Georgia, Athens, GA, USA
• Dr. Mathis & Dr. Lumpkins, Southern Poultry Research, Athens, GA, USA
• Dr. Davis etc., Colorado Quality Research Wilmington, CO, USA
• Dr. Sims, Virginia Diversified Research, Harrisonburg, VA, USA
• Dr. Liu, Life Science Dynamics, Mounds View, MN, USA