In Vitro and In Vivo Evaluation of Therapeutic Effects of NeutraPath™ Against Salmonella Typhimurium

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Salmonella Infections
A Significant Public Health Concern

• In the US, 1.4 million cases of human salmonellosis annually; 100,000 cases due to antibiotic-resistant Salmonella

• Poultry serves as the major reservoir of Salmonellas

• Preharvest Salmonella control is of utmost importance;

• Feed additives are a key pre-harvest measure which can help control Salmonella at the farm level
Colonization in chicken intestinal tract
Central to Entry into the Human Food Chain

- Contamination of Eggshells with Feces
- Carcass Contamination
- Vertical Transmission
- Horizontal Transmission
NeutraPath™
A natural antimicrobial
Antivirulence Strategy May Pinpoint a Paradigm Shift for Pathogen Control

- Toxins
- Adhesins
- Biofilm formation
- Secretion systems (e.g., Type III Secretion systems)
- Cell-to-cell communication
  - Quorum sensing
- Siderophores
- Immune evasion
NeutraPath™

Proven Synergistic Formula

- Bacteriocidal and bacteriostatic effect
- Neutralizing virulence factors
NeutraPath’s minimum inhibitory concentration (MIC)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC (mg/mL)</th>
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<tbody>
<tr>
<td>E. coli K88+</td>
<td>0.625</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>0.625</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>0.625</td>
</tr>
<tr>
<td>P. multocida</td>
<td>0.2345</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>0.078</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>0.625</td>
</tr>
<tr>
<td>B. fragilis</td>
<td>0.625</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>0.156</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.9625</td>
</tr>
</tbody>
</table>
Ability of NeutraPath to Kill Salmonella in SIMULATED GI environment in vitro

Crop
pH ~ 5.2, 30 min

Proventriculus
pH 1.4-2, Pepsin, 45 min

Small Intestine
pH 6.4-6.8, Pancreatin, 2 hour
NeutraPath reduced *Salmonella Typhimurium* bacterial load in the *in vitro* digestion system.
In Vivo Validation

Objectives

To evaluate in vivo effects of NeutraPath on:

• *Salmonella enterica* sv. Typhimurium cecal colonization in broiler chickens;

• Functional integrity of the host’s gut barrier
One-day old male broiler chicks were randomly allocated to one of three groups (n=30 chickens):

- Challenged control with non-treated feed
- NeutraPath supplemented at 0.25%
- NeutraPath supplemented at 0.5%

- Ceca-cecal tonsils removed to evaluate Salmonella recovery
- Serum collected for FITC-d determination

10^6 CFU of live S. Typhimurium
NeutraPath Reduced Prevalence of *Salmonella* Typhimurium in Ceca

**Control**

- 100

**NeutraPath 0.25%**

- 58.33

**NeutraPath 0.5%**

- 66.66
NeutraPath Reduced *Salmonella* Typhimurium Bacterial Load in Ceca

![Bar chart showing S. Typhimurium Log10 cfu/g.](chart.png)

- **Control**: 3.28
- **NeutraPath 0.25%**: 1.44
- **NeutraPath 0.5%**: 1.49

*Note: Values marked with different letters (a, b) indicate statistically significant differences.*
Key Property of *Salmonella*

Ability to invade non-phagocytic epithelial cells

Swiss Army
Salmonella
Typhimurium’s
Virulence Toolkit
- Type III Secretion System (TTSS)
HilA-InvF Axis - Master Regulators For Salmonella Pathogenicity Island 1 (SPI1) TTSS Apparatus Gene Transcription

Eichelberg et al. INFECTION AND IMMUNITY, 1999
NeutraPath down-regulated S. Typhimurium *hilA* and *invF* mRNA expression at subinhibitory concentration (SIC)
NeutraPath’s Suppression of HilA-InvF Axis Further Blocked Expression of Key Downstream Effectors Involved in Invasion

- **sipA**: -23.8
- **sopB**: -17.6
- **sopE**: -20.8
- **sopE2**: -9.8

mRNA expression fold change (NeutraPath/Control)
Tight junction:
Key Target of *Salmonella* Typhimurium
NeutraPath Reduced *In Vivo* Gut Permeability after *Salmonella* Typhimurium Challenge

<table>
<thead>
<tr>
<th>Serum FITC-d (mg/mL)</th>
<th>Control</th>
<th>NeutraPath 0.25%</th>
<th>NeutraPath 0.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>263.44</td>
<td>123.65</td>
<td>150.53</td>
</tr>
</tbody>
</table>

Superscript 'a' indicates statistical significance compared to Control, while 'b' indicates significance compared to NeutraPath 0.25%.
Conclusions

• NeutraPath treatment had the therapeutic potential to reduce *S. Typhimurium* intestinal colonization in broiler chickens;

• Mechanistically, NeutraPath strikingly tuned down SPI-1 TTSS virulence machinery and modified the bacterial ‘behaviors’ to make them more benign;

• As a result of the blockade of SPI-1 virulence development, NeutraPath further preserved gut barrier integrity during *S. Typhimurium* challenge.
Thank you

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