Effects of *Bacillus spp.* probiotics on systemic immunity and intestinal health of weaned pigs experimentally infected with an enterotoxigenic *Escherichia coli*

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Introduction

- Weaning stress
  - Separation from the sow and littermates
  - Different physical environment
  - Sow milk to solid food
- Intestinal structure and function
- Decreased growth performance

(Pluske et al., 1997)
Post-weaning diarrhea by *E. coli* in US

- **Small (< 2000)**
- **Medium (2,000 to 4,999)**
- **Large (>5000)**

**2000**
- Small: 22.1%
- Medium: 32.1%
- Large: 40.7%

**2006**
- Small: 27.8%
- Medium: 45.5%
- Large: 41%

**2012**
- Small: 25.8%
- Medium: 47.1%
- Large: 33.9%

*(USDA NAHMS studies, 2000; 2006; 2012)*
Pathogenesis of *E. coli*

- Ingestion of *E. coli*
- Attachment of the *E. coli* to microvilli
- Production of enterotoxins
- Production/Economic loss
- Diarrhea
- Death
Antibiotics growth promoter

\[ \text{ADG (kg/d)} \]

\[
\begin{align*}
\text{CON} & \quad 0.39 \\
\text{ABX} & \quad 0.45
\end{align*}
\]

\[
\begin{align*}
\text{F:G} & \quad 2.28 \\
\text{ABX} & \quad 2.13
\end{align*}
\]

15% increase
7% decrease

(Cromwell, 2002)
AGP ban in Denmark

Mortality %

Antibiotics consumption, mg/kg pig

AGP ban (weaning pigs)

(Aarestrup et al., 2009)
Antibiotics alternatives - probiotics

- Live microorganisms confer a health benefit
- Modes of action
  - Competition for adhesion sites
  - Direct antagonism
    - Lactic acids – lowering pH
  - Modulation of immune system
    - Anti/pro-inflammatory cytokines
    - Immune cells population

(Kenny et al., 2011)
**Bacillus subtilis**

- Spore-forming *Bacillus spp.*
  - Resistance to harsh environment
  - Long term storage

- Favorable results
  - Reduced incidence of diarrhea
  - Improved intestinal epithelial barrier integrity

*(Bhandari et al., 2008; Yang et al., 2016)*
Objectives

- To investigate the effects of supplementation of *Bacillus* spp. to weaned pigs experimentally infected with an enterotoxigenic F-18 *E. coli*
  - Growth performance
  - Systemic immunity
  - Intestinal health
Materials and Methods

- 36 pigs: 21-d of age, BW = 7.61 ± 0.40 kg
- Individual pens
- 3 dietary treatments
  - Control (CON)
  - CON + *Bacillus subtilis* strain 1 (500 mg/kg*) (PRO1)
  - CON + *Bacillus subtilis* strain 2 (500 mg/kg*) (PRO2)
- 12 replicates/treatment

*500 mg/kg = 1 \times 10^9\text{ cfu/kg diet}
Materials and Methods

- Genotyping: F18 receptor
- Oral inoculation of F18 *E. coli*  
  - LT, STb, SLT-2  
  - $10^{10}$ cfu/dose with 3 doses
- Blood samples  
  - Serum haptoglobin
Materials and Methods

- **Growth performance**
  - Body weight
  - Average daily gain (ADG)
  - Average daily feed intake (ADFI)
  - Feed to gain ratio (FG)

- **Daily diarrhea scores**

- **Duodenum, jejunum, and ileum**
  - Gut morphology (Villi height and Crypt depth)
  - Mesenteric lymph nodes
Statistical analysis

- Mixed Procedure of SAS
- Randomized complete block (BW x Sex)
- Fixed effect: diet
- Random effect: block
- Significance at $P \leq 0.05$ and tendency at $P \leq 0.10$
Results
Body weights

CON  PRO1  PRO2

Body weights, kg

d 7 PI  d14 PI

b  a  ab  b  a  ab
Average daily gain (ADG)

![Graph showing ADG for different groups and time periods.]

- **CON**: Red bars
- **PRO1**: Blue bars
- **PRO2**: Yellow bars

**d 0 to 7 PI**
- CON: 200 g/d
- PRO1: 500 g/d
- PRO2: 400 g/d

**d 7 to 14 PI**
- CON: 800 g/d
- PRO1: 900 g/d
- PRO2: 800 g/d
Crypt depth

- **CON**
- **PRO1**
- **PRO2**

**µm**

- **Duodenum**
- **Jejunum**
- **Ileum**

UCDAVIS
Daily diarrhea score

CON  PRO1  PRO2

*d0  d2  d3  d5  d10  d15  d20*
Frequency of diarrhea (d0 to d20)

- CON
- PRO1
- PRO2

Frequency of diarrhea, ≥ 4
Serum haptoglobin

Haptoglobin, µg/mL

CON  PRO1  PRO2

Haptoglobin, µg/mL

0  500  1,000  1,500  2,000  2,500

d0  d3  d6  d13  d21
Total coliforms

CON  PRO1  PRO2

CFU/mg of sample

Lymph node

0  1000  2000  3000  4000
Jejunal MUC2

Relative mRNA abundance

- CON
- PRO1
- PRO2

- a
- b
- ab
Ileal COX2 and IL1B

CON  PRO1  PRO2

Relative mRNA abundance

COX2

IL1B

UCDAVIS
## Summary

<table>
<thead>
<tr>
<th></th>
<th>PRO1</th>
<th>PRO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>↑</td>
<td>NS</td>
</tr>
<tr>
<td>ADG</td>
<td>↑</td>
<td>NS</td>
</tr>
<tr>
<td>Ileal villi height</td>
<td>↑</td>
<td>NS</td>
</tr>
<tr>
<td>Duodenal crypt depth</td>
<td>↑</td>
<td>NS</td>
</tr>
<tr>
<td>Diarrhea score</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Frequency</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>MUC2</td>
<td>↑</td>
<td>NS</td>
</tr>
<tr>
<td>COX2</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>IL1B</td>
<td>↓</td>
<td>NS</td>
</tr>
</tbody>
</table>
Conclusions

- *Bacillus subtilis* supplementation to *E. coli* challenged weaned pigs had improved:
  - Growth performance
  - Intestinal health

And had reduced:
- Systemic inflammation
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Evonik

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Thank you!