Effects of Bacillus subtilis probiotics on growth performance, diarrhea, and fecal β-hemolytic coliforms 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)

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

Ingestion of *E. coli*

Attachment of the *E. coli* to microvilli

Production/Economic loss

Production of enterotoxins

Diarrhea

Death
Antibiotics growth promoter

[Cromwell, 2002]
AGP ban in Denmark

(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)
Bacillus subtilis reduce gut permeability

- Positive Control
- Bacillus subtilis

Bar graph showing the effect of Bacillus subtilis on transcellular and paracellular permeability. The graph compares the HRP flux (ng/cm²/h) between the two groups. The graph indicates that Bacillus subtilis significantly reduces permeability compared to the positive control.
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

- 48 pigs: 21-d of age, BW = 6.17 ± 0.36 kg
- Individual pens
- 4 dietary treatments
  - Negative Control (NC)
  - Positive Control (PC)
  - CON + Carbadox (50 mg/kg*) (AGP)
  - CON + *Bacillus subtilis* (500 mg/kg*) (PRO)
- 12 replicates/treatment

*500 mg/kg = 1×10⁹ 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
- Fecal samples
  - β-hemolytic coliforms
Materials and Methods

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

- Daily diarrhea scores

- Jejunum and ileum
  - Jejunal tight junction protein mRNA expression
  - Ileal pro-inflammatory cytokine mRNA expression
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

**Graph:**
- **Legend:** NC, PC, AGP, PRO
- **X-axis:** d 0 PI, d 7 PI, d 14 PI, d 21 PI
- **Y-axis:** BW, kg
- **Data points:**
  - d 0 PI: NC = b, PC = a, AGP = b, PRO = b
  - d 7 PI: NC = a, PC = b, AGP = b, PRO = b
  - d 14 PI: NC = ab, PC = c, AGP = bc, PRO = c
  - d 21 PI: NC = a, PC = ab, AGP = bc, PRO = b

*UC Davis*
Average daily gain (ADG)

**ADG, g/d**

- **NC**
- **PC**
- **AGP**
- **PRO**

**Comparison Periods**

- **d 0 to 7 PI**
- **d 7 to 14 PI**
- **d 14 to 21 PI**
- **d 0 to 21 PI**

**Notes**

- Different letters (a, b, c) indicate significant differences among treatment groups within each comparison period.
Daily diarrhea score

NC  PC  AGP  PRO

Diarrhea score

d 0  d 5  d 10  d 15  d 20

UC Davis
Frequency of diarrhea (d0 to d20)

- NC
- PC
- AGP
- PRO

- Score ≥ 4

Legend:
- NC
- PC
- AGP
- PRO

Bar patterns:
a
b
b

Note: The horizontal axis represents the score of diarrhea severity, and the vertical axis represents the percentage of occurrence.
**β - hemolytic coliforms**

Bar chart showing the percentage of β-hemolytic coliforms for different treatments and days post-infection (PI). The chart compares the following treatments:
- **PC** (red bars)
- **AGP** (blue bars)
- **PRO** (yellow bars)

**Days Post-Infection (PI):**
- **d 2 PI**
  - PC: Approximately 70%
  - AGP: Approximately 50%
  - PRO: Approximately 20%

- **d 7 PI**
  - PC: Approximately 70%
  - AGP: Approximately 40%
  - PRO: Approximately 30%

Legends:
- **a** indicates a significant difference compared to the control.
- **b** indicates a significant difference compared to the other treatments at the same day post-infection.
Tight junction protein in jejunum

Relative mRNA abundance

Claudin
Occludin
ZO1
MUC2

NC  PC  AGP  PRO

UCDAVIS
Proinflammatory cytokines in ileum

<table>
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<th>NC</th>
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<th>AGP</th>
<th>PRO</th>
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Relative mRNA abundance
## Summary

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<tr>
<td>ADG</td>
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<td>Feed efficiency (overall)</td>
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<tr>
<td>Diarrhea score</td>
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<tr>
<td>Frequency</td>
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<tr>
<td>β - hemolytic coliforms</td>
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<td>↓</td>
</tr>
<tr>
<td>Claudin</td>
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<tr>
<td>IL6</td>
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<td>COX2</td>
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<tr>
<td>IL1B</td>
<td>NS</td>
<td>NS</td>
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Conclusions

- *Bacillus subtilis* and carbodox supplementation to *E. coli* challenged weaned pigs reduced:
  - Diarrhea score and frequency

and improved:

- Growth performance
- Intestinal health
Acknowledgement

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