Supplementation of *Bacillus amyloliquefaciens* on Growth Performance and Diarrhea Score of Weaned Pigs Experimentally Infected with a Pathogenic *E. coli*

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Introduction

- Postweaning diarrhea
  - Been a threat for the swine industry
  - Cause high morbidity rate in weaning pigs along with huge economical loss
- Postweaning diarrhea is commonly induced by enterotoxigenic *E. coli* (ETEC)

**ETEC pathogenesis**

1. ETEC invades the gut
2. Fimbrial adhesion to epithelial cells of the host
3. Toxins (heat labile toxin and heat stable toxin) are released and invades into epithelial cells
4. Chloride ions are secreted into the lumen
5. Electrolyte-rich fluid are released into the lumen (Van Metre et al., 2008)
6. Watery diarrhea
• Postweaning diarrhea
  • Been a threat for the swine industry
  • Cause high morbidity rate in weaning pigs along with huge economical loss
• Postweaning diarrhea commonly induced by enterotoxigenic *E. coli* (ETEC)
  • Enterotoxins are nonimmunogenic, but immune response can be initiated with antigens present on the fimbrial (surface) proteins

**ETEC pathogenesis**

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5. **Electrolyte-rich fluid are released into the lumen** (Van Metre et al., 2008)
6. **Watery diarrhea**
Antibiotics

• Currently known to be the most effective strategy to alleviate postweaning diarrhea

• However, resistance to antibiotics increased in ETEC and public concern as well
  • Ciprofloxacin increased from 13% in 2005 to 34% in 2009 (Begum et al., 2016)

Probiotics

• Probiotics as potential antibiotic alternative

• Shown to improve growth performance and strengthen immune health of weaning pigs (He et al., 2020; Betancur et al., 2020; Liao and Nyanchoti, 2017)
  • i.e., Lactobacillus spp., Bacillus subtilis, etc.
Bacillus amyloliquefaciens

- Gram-positive aerobic spore forming bacteria
- Usually synthesize polysaccharides and polypeptides
- Rod shaped with flagella
- Induces autophagy by modulating macrophage immunity in vitro (Wu et al., 2017)
- Supplementing *B. amyloliquefaciens* may be a potential probiotics for weaning pigs under diarrheal stress

Annals of clinical microbiology and antimicrobials, 14(1), 1-11.
To investigate effects of dietary supplementation of *Bacillus amyloliquefaciens* (BAM) on diarrhea and growth performance of weaned pigs experimentally infected with pathogenic *E. coli*
Materials & methods

- 50 weaned pigs (7.41 ± 1.35 kg)
  - Around 21 day of age
- 5 treatments (10 pigs per treatment)

<table>
<thead>
<tr>
<th>Sham (-)</th>
<th>CON -</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAM -</td>
<td>0.10% inclusion rate with $10^9$ CFU/kg</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E. coli challenge (+)</th>
<th>CON +</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAM +</td>
<td>0.10% inclusion rate with $10^9$ CFU/kg</td>
<td></td>
</tr>
<tr>
<td>CAR +</td>
<td>0.90% inclusion rate with 50 mg/kg as Carbadox</td>
<td></td>
</tr>
</tbody>
</table>
Materials & methods

Timeline

Pigs were screened for F18 *E. coli* sensitivity prior experimentation

**F18 *E. coli* inoculation**

$10^{10}$ cfu per 3-mL dose in PBS

<table>
<thead>
<tr>
<th>d -7 PI</th>
<th>d 0 PI</th>
<th>d 2 PI</th>
<th>d 7 PI</th>
<th>d 14 PI</th>
<th>d 21 PI</th>
</tr>
</thead>
</table>

**Allotment**

- Pigs housed individually
- *Ad libitum* water and feed

**Euthanasia**

Totaling 28 days (7 d before challenge and 21 days after challenge)

Sample collection

**DAILY**

- Diarrhea score
  - 1 = normal feces
  - 5 = watery diarrhea
- Feed intake

**WEEKLY (d-7, 0, 7, 14, and 21)**

- Body weight
- Fecal swabbing
PROC MIXED of SAS

• Randomized complete block design
• Pig as experimental unit
• Diet and challenge as main effect
• Block as random effect
Results

Bodyweight

![Graph showing bodyweight changes over days post infection for different treatments. The graph includes lines for CON-, BAM-, CON+, BAM+, and CAR+. There is a note with an asterisk indicating significance.](image-url)
Growth performance

d0 to d21 PI

<table>
<thead>
<tr>
<th>ADG, kg</th>
<th>ADFI, kg</th>
<th>Gain:Feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON-</td>
<td>BAM-</td>
<td>CON+</td>
</tr>
<tr>
<td>a</td>
<td>a</td>
<td>c</td>
</tr>
<tr>
<td>ab</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>ab</td>
<td>a</td>
<td>b</td>
</tr>
</tbody>
</table>
Diarrhea frequency

Diarrhea score = DS

DS ≥ 3
- CON-:
- BAM-:
- CON+:
- BAM+:
- CAR+:

DS ≥ 4
- CON-:
- BAM-:
- CON+:
- BAM+:
- CAR+:

Diarrhea frequency vs. DS score

Legend:
- a
- b
- c

Note: DS ≥ 3 and DS ≥ 4 categories are shown with corresponding data points and significance levels.
β-hemolytic coliforms

![Graph showing percentage of β-hemolytic coliforms at d2 PI and d7 PI with different treatments: CON+, BAM+, and CAR+](graph.png)
Conclusions

• Supplementing *B. amyloliquefaciens* tended to enhance growth performance but had limited effects on diarrhea of weaned pigs challenged with *E. coli*

• Further study investigated the systemic immunity of weaned pigs supplemented with *B. amyloliquefaciens* when challenged with *E. coli*
  • Presented in poster presentation “PSII-14 - Supplementation of *Bacillus amyloliquefaciens* on Systemic Immunity of Weaned Pigs Experimentally Infected with a Pathogenic *E. coli*”
Thank you!

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https://animalnutr-ansci.faculty.ucdavis.edu/
References


