Effects of monoglycerides on intestinal morphology and immune responses of weanling pigs experimentally infected with a pathogenic *E. coli*

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Outline

- Gut health and post-weaning diarrhea in pigs
- Monoglycerides
- Experimental design
- Results and conclusions
Gut health of weaning pigs

- Intestinal functions
  - Digest and absorb nutrients
  - Protect the host

- Growth and health of pigs

- Early-life stress
  - Harmful impacts on gut health
Gastrointestinal (GI) function development in pig

Critical Window of postnatal GI Development

- Maternal Immunity
- Plasticity
- Early life stress

GI Barrier Development
- Epithelial barrier and transport functions
- Immune system maturation
- Enteric nervous system

Moeser et al., 2017
Gastrointestinal (GI) function development in pig

Critical Window of postnatal GI Development

Maternal Immunity

Early life stress

Plasticity

Birth 2.5 to 4 weeks 12 to 14 weeks Adult

Normal GI Development

Impaired GI Development
(high early life stress)

Moeser et al., 2018
Weaning stress

- Multifactorial issue
  - Nutritional, physiological, and environmental challenges

- Stressor-induced changes
  - Reduced appetite
  - Induced intestinal dysfunctions
  - Increased exposure and risk to pathogens
  - Post-weaning diarrhea (PWD)
Post-weaning diarrhea (PWD)

- Gastrointestinal disease
  - Economic losses

- Enterotoxigenic *Escherichia coli* (ETEC)
  - F4 (K88) and F18 *E. coli*
  - Enterotoxins
    - Secretory diarrhea
Post-weaning *E. coli* diarrhea

1. Ingestion of ETEC
2. Attachment of ETEC to receptors using fimbriae
3. Colonization and release of toxins
4. Increased gut permeability (water and electrolytes into intestine)
5. Watery diarrhea & dehydration

- Productivity
- Animal welfare
- Mortality

Kim et al., 2022
**E. coli** challenge and diarrhea

**Non Ruminant Nutrition**

Effects of an F18 enterotoxigenic *Escherichia coli* challenge on growth performance, immunological status, and gastrointestinal structure of weaned pigs and the potential protective effect of direct-fed microbial blends

Spenser L. Becker, Qingyun Li, Eric R. Burrough, Danielle Kenne, Orhan Sahin, Stacie A. Gould, and John F. Patience

Figure 1. Effects of treatment on the daily fecal score of pigs challenged with F18 ETEC. NC (n = 10); PC (n = 9); DFM1 = PC + direct-fed microbial 1 (n = 8; three strains of *Bacillus amyloliquefaciens*; 7.5 × 10⁶ cfu/g of feed); DFM2 = PC + direct-fed microbial 2 (n = 7; two strains of *B. amyloliquefaciens* and one strain of *Bacillus subtilis*; 1.5 × 10⁷ cfu/g of feed). Supplementation rates were based on manufacturer’s recommendations (Danisco Animal Nutrition). P (NC vs. PC; day postinoculation [dpi] 3) < 0.001, P (PC vs. DFM1, DFM2; dpi 3) > 0.10, P (all treatments; dpi 10) > 0.10.

*Fecal score: 0 = solid; 1 = semi-solid; 2 = semi-liquid; 3 = liquid (≥ 2 was considered diarrhea)*
E. coli challenge and performance

Dietary supplementation of botanical blends enhanced performance and disease resistance of weaned pigs experimentally infected with enterotoxigenic Escherichia coli F18

Braden T. Wong,† Sangwoo Park,† Lauren Kovanda,† Yijie He,† Kwangwook Kim,† Shiyou Xu,† Christopher Lingga,† Monika Hejna,‡ Emma Wall,‡ Ravichandran Sripathy,§ Xunde Li,† and Yanhong Liu*
Antibiotics and pharmacological dose ZnO

- Prevent and treat PWD
  - Antimicrobial effects
  - Nutrients availability

- Public health risk and concern
  - Antimicrobial resistance
  - Environmental transmission
    - Prohibition of AGPs (Jan 2017, FDA)
    - Prohibition of pharmacological dose ZnO (June 2022, EU)

Public Health Agency of Canada, 2017
Monoglycerides (MGs)

- **Glycerol** linked to **fatty acid** (esterification)
  - Short chain and medium chain fatty acids
- **Natural compound**
  - Used in food processing and production
- **Amphiphilic nature**
  - Hydrophobic & hydrophilic
  - **Antimicrobial activity**

Jackman et al., 2022
Antimicrobial effects of MGs (*in vitro*)

- Incorporate into the lipid membrane of microorganisms and **change the permeability**

Hyldgaard et al., 2012; Joshua et al., 2020
Additional benefits of MGs

- Strong covalent bond

<table>
<thead>
<tr>
<th></th>
<th>Organic acid</th>
<th>Monoglycerides</th>
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</thead>
<tbody>
<tr>
<td>Antimicrobial</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Non-corrosive</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Non-volatile</td>
<td>-</td>
<td>✓</td>
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<tr>
<td>Heat stable</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Neutral taste odor</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>pH-independent</td>
<td>-</td>
<td>✓</td>
</tr>
</tbody>
</table>

- Easy to handle and use
Objective

- Effects of a mixture of monoglycerides on weaned pigs experimentally infected with a pathogenic *Escherichia coli* (E. coli) F18

  ✓ Growth performance
  ✓ Diarrhea
  ✓ Intestinal health
  ✓ Immune responses
Animals & experimental design

- **Animals**
  - 60 weaned pigs (initial BW = 6.5 ± 0.74 kg; 21 d old)
    - Individual house (15 replications/treatment)

- **4 dietary treatments**
  - Corn-soybean meal-based diet (Control)
  - Control + 0.3% monoglycerides
  - Control + 3000 ppm zinc oxide (ZnO)
  - Control + 50 mg/kg of antibiotic (carbadox)

- **2-phase feeding** (2 weeks/phase; overall 4 weeks)
Timeline and data collection (I)

- **Daily fecal scores**
  - Score 1 to 5 = firm feces to watery diarrhea
- **Percentage of β-hemolytic coliforms** in feces
- **Growth performance** (ADG, ADFI, and G:F)

All pigs were orally inoculated with pathogenic *E. coli* F18 ($10^{10}$ CFU/dose)
β-hemolytic coliforms (feces)

Columbia blood agar
(β-hemolytic coliforms)

MacConkey agar
(Lactose-fermenting bacteria)
Timeline and data collection (II)

- Serum **acute phase protein level** (d 0, 2, 5, and 21 PI)
  - C-reactive protein and haptoglobin
- Intestinal **morphology** and **immune-related gene expression**
  - d 5 PI (6 pigs/treatment) and d 21 PI (9 pigs/treatment)

All pigs were orally inoculated with pathogenic *E. coli* F18 (10^{10} CFU/dose)
Statistical analysis

- PROC MIXED of SAS
  - Randomized complete block design (block: BW)
  - Experimental unit: pig
  - Fixed effect: dietary treatment

- Chi-square test
  - Frequency of diarrhea
Daily fecal score

$*P < 0.05$
$\#P < 0.10$

*Fecal score = 1, firm feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; 5, watery diarrhea*
Frequency of diarrhea (overall)

Incidence of diarrhea, ≥ 3
- Control
- Monoglycerides
- ZnO
- Antibiotics

Severity of diarrhea, ≥ 4
- Control
- Monoglycerides
- ZnO
- Antibiotics
β-hemolytic coliforms (feces)
Acute phase proteins (serum)

C-reactive protein

Haptoglobin
Duodenum goblet cell number

![Bar chart showing goblet cell number per villus at d 5 PI for different treatments: Control, Monoglycerides, ZnO, and Antibiotics. Each bar is labeled with letters indicating statistical significance.](image-url)
Duodenum villi area and height

Villi area

Villi height

\[ \text{Villi area} \]

\[ \text{Villi height} \]

\[ \text{Control, Monoglycerides, ZnO, Antibiotics} \]
Duodenum VH:CD
Ileum CD & VH:CD

Crypt depth

VH:CD

\[ P = 0.064 \]
Duodenum villi area and height (d 21 PI)

Villi area

Villi height

µm²

µm

d 21 PI

Control   Monoglycerides   ZnO   Antibiotics

ab   b   a   b

b   b   a   b
Ileum mucosa gene expression (d 5 PI)
Ileum mucosa gene expression (d 21 PI)
Body weight
Growth performance

Average daily gain

Average daily feed intake
Conclusions

- **Monoglycerides** supplementation
  - Reduce the diarrhea severity
  - Have a positive effect on the intestinal morphology
  - Modify the intestinal and systemic inflammation of weaned pigs infected with ETEC F18
Acknowledgements

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https://animalnutr-ansci.faculty.ucdavis.edu/
Greatly appreciate your attention!