Gastrointestinal ecosystem and immunological responses in pigs after weaning fed liquid diets containing whey permeates fermented with different LAB

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Outline:

› Backgrounds
› Objectives of study
› Experimental design
› Findings
Backgrounds

› Feeding liquid diet:
  »»» Keep high and regular feed and water intake post-weaning

« « « Risk for enteropathogenic infection when trough system is used
  (soaking period allows proliferation of *enterobacteriaceae*)
Backgrounds

› Whey permeate (WP):
  » Byproduct of cheese-making ≈ rich in lactose
  » Potentially synbiotic fermented product that may be added to the liquid diet:
    o Prevent the overgrowth of enterobacteriaceae during feeding
    o Exert both prebiotic and probiotic effect
Backgrounds

»»» Beneficial effects of fermented products for gut health ≈ lactic acid bacteria (LAB)

««« The effect of LAB ≈ species and strain specific
Objective of study

- Investigate the effect of feeding liquid diets containing WP fermented with different LAB species on GIT microbial populations and mucosal immune responses of *E. coli* F4 infected pigs after weaning.

**Hypotheses:**
Feeding fermented-WP may balance gut microbiota and modulate mucosal immunity of pigs in response to ETEC invasion.

The effect of fermented-WP on gut microbiology and immunology may depend on the species of LAB used to ferment WP.
# Experimental design

<table>
<thead>
<tr>
<th></th>
<th>INF−WP−</th>
<th>INF+WP−</th>
<th>INF+W P+</th>
<th>INF+W P+ LAB1</th>
<th>INF+W P+ LAB2</th>
<th>INF+W P+ LAB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diets</td>
<td>n=6</td>
<td>n=8</td>
<td>n=10</td>
<td>n=8</td>
<td>n=10</td>
<td>n=10</td>
</tr>
<tr>
<td>Control diet</td>
<td>Control diet</td>
<td>Control diet</td>
<td>Diet + non-fermented WP</td>
<td>Diet + WP fermented with <em>S. thermophilus</em>/<em>L. bulgaricus</em></td>
<td>Diet + WP fermented with <em>L. plantarum</em></td>
<td>Diet + WP fermented with <em>W. viridescens</em></td>
</tr>
<tr>
<td><em>E. Coli</em> F4</td>
<td>•</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>E. Coli F4</strong> challenge</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
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</thead>
<tbody>
<tr>
<td>Weaning day</td>
<td>^</td>
<td></td>
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<tr>
<td><em>E. Coli</em> F4 challenge</td>
<td>^</td>
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</tr>
<tr>
<td>Faecal sampling</td>
<td>^</td>
<td>^</td>
<td>^</td>
<td>^</td>
<td>^</td>
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<td>^</td>
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<tr>
<td>Killing and sampling (digesta &amp; SI)</td>
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</tr>
</tbody>
</table>
## Composition of the experimental diets (%)

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>WP groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP (Variolac 830)</td>
<td>0</td>
<td>#</td>
</tr>
<tr>
<td>Barley</td>
<td>20.000</td>
<td>20.000</td>
</tr>
<tr>
<td>Wheat</td>
<td>48.200</td>
<td>41.200</td>
</tr>
<tr>
<td>Dehulled toasted soybean meal</td>
<td>16.690</td>
<td>17.610</td>
</tr>
<tr>
<td>Animal fat</td>
<td>3.000</td>
<td>3.000</td>
</tr>
<tr>
<td>Soy protein concentrate</td>
<td>3.000</td>
<td>3.250</td>
</tr>
<tr>
<td>Potato protein</td>
<td>5.000</td>
<td>5.000</td>
</tr>
<tr>
<td>Other*</td>
<td>4.110</td>
<td>3.940</td>
</tr>
</tbody>
</table>

#WP included in the diet = 64 g WP per kg diet

*L-Lysine HCL, DL-Methionine, L-Threonine, L-Tryptophan, L-Valine, Monocalcium phosphate, Calcium carbonate, 38% Ca, Sodium chloride, Natrupalos 5000 (100g/t), Vitamin and mineral premix*
Preparation of inoculum

-80°C stock

Streaked on MRS plate, incubated at 37°C overnight, aerobic

Picked single colony, inoculated in MRS broth, incubated at 37°C, overnight, aerobic
Preparation of fermented WP

1. 70 g WP + 1 g yeast extract dissolved in 1000 mL water
2. Pasteurized at 80°C, 20 min
3. Let the suspension cool, and adjusted to pH 6.0
4. Inoculated with 4 mL of the LAB-inoculated MRS broth
5. Incubated at 37°C, 24 h, aerobic, to reach pH 4.4 and 10⁸ cfu LAB/mL
6. Store at 4°C
Preparation of diets

Immediately before feeding

INF−WP−

INF+WP−

INF+WP+

INF+WP+LAB1

INF+WP+LAB2

INF+WP+LAB3

Dry feed = 1 wt

Water = 2.5 v

Water = 1.6 v

Dry feed = 1 wt

WP fermented with LAB1 = 0.9 v

WP fermented with LAB1 = 0.9 v

WP fermented with LAB1 = 0.9 v

Water = 1.6 v

Water = 1.6 v

Water = 1.6 v

Non fermented WP = 0.9 v

INF−WP−

INF+WP−

INF+WP+

INF+WP+LAB1

INF+WP+LAB2

INF+WP+LAB3
Performance of pigs

Feed intake, d1–11

Weight gain, d1–11

SE=691; P=0.20

SE=280; P<0.001

gram
Faecal-haemolytic *E. coli*

- $P_{\text{treatment}} = 0.55$
- $P_{\text{day}} < 0.001$
- $P_{\text{treatment} \times \text{day}} = 0.48$
- SE = 0.26

*Log CFU/g*

Day of experiment

- INF–WP–
- INF+WP–
- INF+WP+
- INF+WP+LAB1
- INF+WP+LAB2
- INF+WP+LAB3
Faecal-LAB

Log CFU/g

Day of experiment

INF–WP– INF+WP– INF+WP+ INF+WP+LAB1 INF+WP+LAB2 INF+WP+LAB3

$P_{\text{treatment}} = 0.49$

$P_{\text{day}} < 0.001$

$P_{\text{treatment} \times \text{day}} = 0.53$

$SE = 0.21$
**F-Total coliform**

- **Log CFU/g**
- **Day of experiment**
- $P_{\text{treatment}}=0.89$
- $P_{\text{day}}<0.001$
- $P_{\text{treatment} \times \text{day}}=0.76$
- SE=0.19

**F-C. perfringens**

- **Log CFU/g**
- **Day of experiment**
- $P_{\text{treatment}}=0.10$
- $P_{\text{day}}<0.001$
- $P_{\text{treatment} \times \text{day}}=0.36$
- SE=0.55

**F-Yeast**

- **Log CFU/g**
- **Day of experiment**
- $P_{\text{treatment}}=0.59$
- $P_{\text{day}}=0.01$
- $P_{\text{treatment} \times \text{day}}=0.17$
- SE=0.41

**F-Total anaerobic bacteria**

- **Log CFU/g**
- **Day of experiment**
- $P_{\text{treatment}}=0.74$
- $P_{\text{day}}=0.01$
- $P_{\text{treatment} \times \text{day}}=0.78$
- SE=0.09
**Digesta-total coliform**

- **Log CFU/g**
- **P_{treatment} = 0.06**
- **P_{segment} < 0.001**
- **P_{treat*seg} = 0.68**
- **SE = 0.28**
Dige sta-LAB: *coliform* ratio

![Graph showing coliform ratio in different segments of the digestive tract.](image)

- **Stomach**: INF–WP–, INF+WP–, INF+WP+, INF+WP+LAB1, INF+WP+LAB2, INF+WP+LAB3
- **Distal-SI**: INF–WP–, INF+WP–, INF+WP+, INF+WP+LAB1, INF+WP+LAB2, INF+WP+LAB3
- **Caecum**: INF–WP–, INF+WP–, INF+WP+, INF+WP+LAB1, INF+WP+LAB2, INF+WP+LAB3
- **Mid-colon**: INF–WP–, INF+WP–, INF+WP+, INF+WP+LAB1, INF+WP+LAB2, INF+WP+LAB3

- \( P_{\text{treatment}} = 0.07 \)
- \( P_{\text{segment}} < 0.001 \)
- \( P_{\text{treat \times seg}} = 0.89 \)
- \( SE = 0.06 \)
**Digesta-LAB**

- $P_{treatment} = 0.56$
- $P_{segment} < 0.001$
- $P_{treatment*segment} = 0.33$
- $SE = 0.14$

**Digesta-Yeast**

- $P_{treatment} = 0.43$
- $P_{segment} = 0.02$
- $P_{treatment*segment} = 0.19$
- $SE = 0.26$

**Digesta-Total anaerobic bacteria**

- $P_{treatment} = 0.53$
- $P_{segment} < 0.001$
- $P_{treatment*segment} = 0.11$
- $SE = 0.16$
Mucosal IgA – 90%SI

SE = 0.06; P = 0.02

mg/g

INF–WP– | INF+WP– | INF+WP+ | INF+WP+LAB1 | INF+WP+LAB2 | INF+WP+LAB3

ab | b | b | ab | b | b
Mucosal IgM – 90%SI

SE=0.34; P=0.11
Biliary IgA and IgM

**IgA**
- SE = 1.70; $P = 0.08$

**IgM**
- SE = 0.79; $P = 0.49$

The graphs show the levels of IgA and IgM in different conditions, with the bars representing the concentrations in mg/dL.
**Gene expression level**

**IL-10**

- INF-WP-
- INF+WP-
- INF+WP+LAB1
- INF+WP+LAB2
- INF+WP+LAB3

- Fold change
- SE=0.16; P=0.02

**Cox-2**

- INF-WP-
- INF+WP-
- INF+WP+LAB1
- INF+WP+LAB2
- INF+WP+LAB3

- SE=0.7; P=0.17
Gene expression level

**TNF-α**

SE = 0.26; *P* = 0.10

**IL-2**

SE = 0.25; *P* = 0.55
Summary of results

› Feeding fermented-WP had no sig. impacts on faecal microbial population across the sampling days.

› Fermented-WP tended to reduce *coliform* bacteria and increase LAB:*coliform* ratio in the GIT digesta, but had no impact on LAB, yeast, and total anaerobic bacteria.

› Feeding WP or fermented-WP tended to reduce production of IgA and IgM in the distal intestine and bile of the challenged pigs.

› Treatments had impact on the gene expression level of IL-10, but not on Cox-2, TNF-± and IL-2. The effect of LAB on IL-10 seemed to be species dependent.

› Treatments had no impact on the intestinal dimension of pigs (not shown).
Discussion of results

› Higher LAB: *coliform* ratio in digesta of pigs fed fermented-WP was most likely ascribed by the lower counts of *coliform* bacteria.

>>> Feeding fermented-WP reduced the growth of *coliform* resulting in less immune-stimulation (less Cox-2 expression and IgA and IgM).

Feeding fermented-WP maintained GIT microbial ecosystem and modulated the mucosal immune responses of *E. coli* F4 challenged pigs.

The effect of LAB on host immune systems seems to be species dependent.
Acknowledgment

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› Sarmauli I. Manurung (National Veterinary Institute, Technical University of Denmark)
Thank you for your attention