Canadian Alternatives for Dietary Antibiotics as Growth Promotants

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Less developed digestive system.
Poor feed intake → negative effects.
Hypersensitivity to feed antigens.
Less developed immune system.
Feed Additives with Potential to Promote Gut Health and Function

- Organic Acids
- Probiotics
- Prebiotics
- ZnO, CuSO₄
- Plasma Proteins
- Essential Oils
- Enzymes
- Resistant Starch
- Herbs and Spices
- Specific Antibodies
- Oligosaccharides-COS
- MC Fatty Acids

- Lysozyme
- Antimicrobial Peptides
- Bioactive Peptides
- Nucleotides
- Yeast cell Compounds
- Bacteriophages
- Activated Charcoal
- Silaceous Earths
- Rare Earth Elements
- Tannins/Polyphenolics
- Toxin binders
Dietary Additives

↓ Pathogen Colonization
↑ Beneficial Bacteria

↓ Immune Stimulation

↓ Gut Damage
Maintains Gut Structure & Function

↓ Diarrhea Incidences

Spare Nutrients for Growth

Improved Digestive & Absorptive Capacity

Improved Performance and Gut Health Outcomes
E. coli K88 Disease Challenge Model
→ mode of action studies

Small intestinal segments
→ measures fluid balance

Ussing Chambers → gut integrity and function
**Modes of Action of Probiotics**

**Antimicrobial activity**
- Decrease luminal pH
- Secrete antimicrobial peptides
- Inhibit bacterial invasion
- Block bacterial adhesion to epithelial cells

**Enhancement of Barrier Function**
- Increase mucus production
- Enhance barrier integrity

**Immunomodulation**
- Effects on epithelial cells
- Effects on dendritic cells
- Effects on monocytes/macrophages
- Effects on lymphocytes

Probiotics reduced ileal attachment and fecal shedding of ETEC K88 in weaned pigs challenged with ETEC.

E. coli-based probiotics inhibited growth of pathogenic E. coli K88+ (ETEC) and potato starch acted synergistically to reduce the effects of ETEC infection.
Effect of an *E. coli* Probiotic on Diarrhea Score (A) and Mucosal-Attached ETEC (B) in Challenged Piglets

- PRO tended ($P = 0.086$) to support better growth performance.
- PRO reduced *E. coli* K88 colonization of mucosal surface $\rightarrow \downarrow$ diarrhea
Properties of a Prebiotics

- Resistant to digestion in the upper GIT - resistant to acid and enzymatic hydrolysis.
- A selective substrate for the growth of beneficial bacteria in the colon.
- Induce luminal or systemic effects that are beneficial to host health.

How Prebiotics Increases Resistance to Infection

Prebiotic

well-balanced commensal bacteria

fermentation
pH reduction

SCFA

anti-adhesive

Lumen

Pathogen

Intestinal epithelium

Dendritic cells

Immunomodulation

T cells

B cells

Cytokine regulation

Metabolic regulation

Improve villi growth, crypt development, tight junctions and mucin production

Lectin receptors

Pourabedin and Zhao. 2015. FEMS microbiology letters. http://dx.doi.org/10.1093/femsle/fnv122
Supplementing Resistant Starch in pig Starter Diets at 7% Reduced FS to Same Level as an Antibiotic

- RPS increases Lactobacilli and bifidobacteria in the colon (Wang et al., 2002)
- 14% RSP reduced microbial richness and diversity in the colon – associated with digestive abnormalities (Bhandari et al., 2008).
Resistant Potato Starch Affects Lower Gut Physiology and Reduces Scours in Piglets

Heo et al., 2014; J. Anim. Sci. 92:3895-3902
SYNBIOTICS

PROBIOTICS

PREBIOTICS

Improves survival and implantation of live microbial dietary supplements (Gibson and Roberfroid, 1995)

• *Lactobacilli* + inulin / FOS / GOS / etc.
• *Bifidobacteria* + inulin / FOS / GOS / etc.
• Colicin-producing *E. coli* + raw potato starch

Bhandari et al., 2008; Trevisi et al., 2008; Lee and Salminen, 2009; Nemcova et al., 1998; Ohima et al., 2016

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Oral Administration of Egg Yolk Antibody Powder Against ETEC Infection in Piglets

*antibody titre; **anti-fimbrial antibodies removed

- High titre antibodies prevented bacterial adhesion
- Anti-fimbrial antibodies were the active components

Yokoyama et al., 1992; Infect. Immun. 60:998-1007
EYA Improves Gut Health Outcomes in ETEC-Challenged Piglets

24 hr Scour scores

48 hr Scour Scores

VH:CD

d 7 ETEC Shedding

Owusu-Asiedu et al., 2003; J. Anim. Sci. 81:1781
Egg Yolk Antibodies

- Effectively control incidences of diarrhea caused by a specific pathogen.
- Work best when target pathogen is causative agent for diarrhea.
- Ability to withstand feed processing conditions.

Dietary EYA enhances gut barrier function in *E. coli* challenged piglets – Kiarie et al. (2009)
Prebiotic Effects of Feed Enzymes

K88 E. coli

Lactobacillus

E. coli attachment to intestinal surface glycoproteins

Lactobacilli and factors produced by lactobacilli block E. coli attachment sites
Effect of Carbohydrases Addition on Ileal Lactobacilli and Lactic Acid in Piglets

Kiarie et al., 2007; J. Anim. Sci. 85:2982
Enzyme hydrolysis products enhanced fluid absorption in piglet intestines

Kiarie et al., 2009; Anim. Sci. J. 81:63
Enzyme Hydrolysis Products Reduced Fecal Scores in ETEC Infected Piglets.

Kiarie et al., 2009
Effect of Enzyme Hydrolysis Products on Intestinal Environment After ETEC†

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Hydrolysis Products</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid*</td>
<td>44.3</td>
<td>87.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Total organic acids*</td>
<td>50.1</td>
<td>95.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Stomach pH</td>
<td>3.86</td>
<td>2.62</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*mmol/ L; †24 h after ETEC Challenge.

Kiarie et al., 2009; Can. J. Anim. Sci. 89:34.
Use of Dietary Nucleotides

- Are building blocks of:
  a) Nucleic acids (RNA & DNA)
  b) Cellular energy (ATP)
  c) Co-enzymes

- Enhance structural growth, especially the maturation of the immune system and gut mucosa.
Dietary Nucleotide Rich Yeast Extract Improved Piglet Performance*

*NRYE added at 0.1%; Study lasted 4 weeks post-weaning.

Waititu et al., 2015
Dietary Nucleotide Rich Yeast Extract Favored Proliferation of Beneficial Gut Bacterial

**Beneficial bacteria**

- **Lactobacillus**
  - $P = 0.002$
  - Control: Red, NRYE: Blue
  - Clean room: a, Unclean room: b

- **Clostridium cluster IV**
  - $P = 0.026$
  - Control: Red, NRYE: Blue
  - Clean room: a, Unclean room: b

**Pathogenic bacteria**

- **Enterococcus**
  - $P = 0.041$
  - Control: Red, NRYE: Blue
  - Clean room: a, Unclean room: b

- **Enterobacteriaceae**
  - $P = 0.001$
  - Control: Red, NRYE: Blue
  - Clean room: a, Unclean room: b
Effect of Dietary NRYE on Gut Morphology in LPS Challenged Piglets

All supplements increased villus height

Effect of Dietary NRYE on Ileal IFN-γ & TNF-α in LPS Challenged Piglets

Dietary YE supplementation downregulated pro-inflammatory cytokines.

Effects of Dietary Nucleotide Rich Yeast Extracts in Piglets

- Improved intestinal morphology in LPS challenged piglets.
- Positive modulation of the immune system → reduced gut damage.
- Favored proliferation of beneficial bacteria while suppressing harmful ones.

Results of U of M Studies by Waititu et al., 2016a,b
Antimicrobial Peptides

- **Epidermal growth factor** → 124 µg/L in sow milk
  - Oral administration has benefits in piglets:
    - Enhance intestinal mucosal healing → e.g. recovery from rotavirus infection
    - Direct administration of recombinant EGF is too costly for commercial use.

- **Bacteriocins**
  - **Colicins** → produced by *E. coli*
  - Shown to inhibit growth of enterotoxigenic *E. coli* (Setia et al., 2009) and reduce diarrhea in piglets.
Dietary EGF Improves Gut Structure and Function in Piglets

- Improved gut structure.
- Improved digestive enzyme activities.
- Improved immune responses.
- Enhance nutrient transport systems.
- Enhance barrier function.

Kang et al., 2010; Bedford et al., 2012, 2014, 2015
Role of Plant Polyphenolic Compounds
- preliminary observations -

- Potential role for red osier dogwood:
  - High content of polyphenolic compounds.
  - These compounds are known to have many beneficial effects:
    - Antioxidants
    - Antimicrobial
    - Immune modulation
Results of a U of M Study with Piglets

- Use of an *E. coli* challenge model
  - 7 days feeding of diets; 7 days observation.
  - Performance and indicators of oxidative stress.

<table>
<thead>
<tr>
<th>Villous height:Crypt Depth</th>
<th>PC</th>
<th>NC</th>
<th>ROD 2%</th>
<th>ROD 4%</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1.7</td>
<td>1.5</td>
<td>1.3</td>
<td>1.1</td>
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*P = 0.07 NC vs. ROD*
Dietary ROD Reduced Oxidative Stress and enhanced Antioxidant Capacity in *E. coli* Challenged Piglets

<table>
<thead>
<tr>
<th></th>
<th>Ileum MDA, % of control</th>
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<th>Ileum SOD, % of control</th>
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<tbody>
<tr>
<td>UC</td>
<td>a</td>
<td>UC</td>
<td>a</td>
</tr>
<tr>
<td>NC</td>
<td>b</td>
<td>NC</td>
<td>b</td>
</tr>
<tr>
<td>DW2</td>
<td>a</td>
<td>DW2</td>
<td>a</td>
</tr>
<tr>
<td>DW4</td>
<td>a</td>
<td>DW4</td>
<td>a</td>
</tr>
<tr>
<td>PC</td>
<td></td>
<td>PC</td>
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Manipulating Dietary Protein Content

The evils of protein fermentation in the intestines of piglets

- Indigestible protein
  - Source of N for bacteria
    - Fecal N
  - Fermented for energy
    - Decarboxylation
    - Deamination
    - Elimination
      - Monoamines
      - Polyamines
      - BCFA
      - SCFA
      - NH3
      - H2S
      - Phenols/indoles
      - Receptor ligands, cell proliferation, cytotoxicity, buffering
      - Tight junction failure, reduced barrier function
        - => Inflammation, translocation of pathogens

Sources: Jérôme Bindelle; https://orbi.ulg.ac.be/bitstream/
E. coli K88 population was lower in ileal digesta of piglets fed a low CP diet 3 days (A) and 7 days (B) after challenge with ETEC K88.
A LCP diet acted Synergistically with a Probiotic to Support Better ADG

Bhandari et al., 2010
Ileal Mucosa-attached ETEC K88 and Diarrhea Scores in ETEC Challenged Piglets

|                | High CP Diet |          |          | Low CP Diet |          |          |
|                | CON  | AGP  | PRO |          | CON  | AGP  | PRO |
| ETEC*          | 5.25 | 2.96 | 2.72 |            | 3.90 | 2.47 | 2.51 |
| Diarrhea       | 2.38 | 1.55 | 1.42 |            | 1.75 | 1.05 | 1.17 |

*values are log_{10} cfu/g of tissue

The LP diet worked synergistically with the probiotic to improve growth performance and reduce *E. coli* K88 colonization of the mucosal surface in piglets after weaning.

*Bhandari et al., 2010*
Husbandry Practices and Gut Health in Piglets are Profoundly Interrelated

- Nutrition and management practices.
- Hygiene standards and disease prevention.
- Animal welfare considerations (e.g. space allowance & temperature).
Husbandry Practices that Affect Gut Health in Piglets

- Feed Intake
- Stress
- Sanitation
- Disease

Growth / Gut Health
Concluding Remarks

- Many additives available in Canada with potential to replace antibiotics.
  - Some are better characterized than others
- Strategic products involving multiple additives are likely to be more effective.
  - Synergistic effects
  - Mode of action for each additive.
- Dietary manipulation plus robust husbandry practices are critical.
Summary and Conclusions

- *husbandry practices vs. gut health*

- Husbandry practices are important determinants of piglet performance.
  - Feeding and nutrition.
  - Management practices and animal welfare.
  - Biosecurity and disease prevention.

- Must be considered as a critical piece in the overall strategy of raising pigs without in-feed antibiotics.
  - Related to gut structure and function!
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