Supplementation of Oligosaccharide-based Polymer Enhanced Growth and Disease Resistance of Weaned Pigs by Modulating Intestinal Integrity and Systemic Immunity

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Outline

- Challenges in swine industry
- In-feed antibiotics & potential alternative methods
- Research findings & implications
Global population growth: Feeding the world in 2050

Source: United Nations, Department of Economics and Social Affairs, Population Division (2014). World Urbanization Prospects: The 2014 Revision, custom data acquired via website
Can we meet the rising global food demand?

In the year 2050, 70% of the world population will require more food, and 70% of this food must come from efficiency-improving technology.

Source: World Agriculture: Towards 2015/2030. Summary Report (https://www.fao.org/3/y3557e/y3557e.pdf)
5 challenges that will influence food production towards 2050

- Increase food production per acre
- Climate change
- Farmland limitation
- Improving food quality
- Natural resources

Source: www.agrocares.com/en/news/5-challenges-food-production
Growth in global protein demand

|                | 2005 | 2050 |
|----------------|------|------|
| Beef           | 64   | 106  |
| Pork           | 100  | 143  |
| Poultry        | 82   | 181  |
| Eggs           | 62   | 102  |

Source: Food and Agriculture Organization of the United Nations, ESA Working Paper No. 12-03, p. 131
Life cycle of a market pig

- **Farrowing**: 3 months, 3 weeks and 3 days (114 d)
- **Weaning**: 6 to 8 weeks (42 to 56 d)
- **Growing cycle**: 16 to 17 weeks (115 to 120 d)
- **Finishing**: 3 weeks (21 d)
- **To the consumer**: 3 months, 3 weeks and 3 days (114 d)
Weaning stress

- Environmental changes
- Abrupt transition of diet
- Transportation stress
- Increase the risk of exposure to disease
Gastrointestinal (GI) tract development during weanling

Critical Window of postnatal GI Development

- Epithelial barrier and transport functions
- Immune system maturation
- Enteric nervous system

Maternal Immunity

Birth

2.5 to 4 weeks

12 to 14 weeks

Adult

Source: Moeser et al., 2017
Post-weaning diarrhea in pigs

• One of the most serious threats for the swine industry

• Usually associated with proliferation of enterotoxigenic *E. coli* (ETEC)
  ✓ F4 (K88)
  ✓ F18
Post-weaning ETEC diarrhea morbidity

Source: USDA, Swine 2012 Part III: Changes in the U.S. Swine Industry, 1995–2012

E. coli diarrhea

% of sites (US)

2000: 24%
2006: 31.8%
2012: 32.4%
ETEC pathogenesis

Ingestion of ETEC

Attachment of ETEC to receptors through fimbriae

Colonization and release of toxins

Increase gut permeability (water and electrolytes into intestine)

Small intestinal epithelial cells

Diarrhea

=ETEC (Enterotoxigenic E. coli)

=Toxins
Swine (Livestock) production technologies

- Genetics
- Reproduction
- Nutrition
- Manage-ment
- Health

- Macro-nutrients
- Micro-nutrients
- Non-nutrient feed additives
Antibiotics use in livestock

Antimicrobial substances active against bacteria

• Disease prevention
• Disease treatment
• Growth promotion

Efficacy of antibiotics as growth promoters for weaned pigs (7-25 kg)

Source: Zimmerman, 1986
Antibiotics as growth promoter in animal diets poses risk

- Emergence of antibiotic resistance
- Banned in the European Union since 2006
- Also restricted in the United States since 2017

Source: https://fairfarmsnow.org
Trace levels of antibiotics: A global health hazard

- Manure
- Surface water
- Soil
- Air
- Dust
- Farm environment

Source: Harbarth et al, 2015
Adverse effects of trace levels of antibiotics

- Toxicity
- Mutagenicity
- Carcinogenicity
- Hypersensitivity
- Antibiotic resistance

Young animals are more sensitive!

Delay the growth & recovery from diseases

Source: Onegreenplanet.org
Keyword occurrence in academic papers: “Antibiotic alternatives”

Source: Web of Science
ETEC pathogenesis

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Ingestion of ETEC

Small intestinal epithelial cells

Diarrhea

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Ingestion of *E. coli*

Small intestinal epithelial cells

Attachment of *E. coli* to receptors through fimbriae

Colonization and release of toxins

Increase gut permeability (water and electrolytes into intestine)

Diarrhea

- Organic acids
- Antimicrobial peptides
- Bacteriophages

Ingestion of *E. coli* = ETEC (Enterotoxigenic *E. coli*)

= Toxins

Alternative antimicrobial approaches against ETEC

Organic acids

Antimicrobial peptides

Bacteriophages

Small intestinal epithelial cells
Ingestion of *E. coli*

- Attachment of *E. coli* to receptors through fimbriae
- Colonization and release of toxins
- Increase gut permeability (water and electrolytes into intestine)

Diarrhea

**Attachment of *E. coli* to receptors through fimbriae**

- Oligosaccharides
- Direct-fed microbials
- Prebiotics

=ETEC (Enterotoxigenic *E. coli*)

=Toxins
Ingestion of E. coli

Small intestinal epithelial cells

Attachment of E. coli to receptors through fimbriae

Colonization and release of toxins

- Direct-fed microbials
- Oligosaccharides
- Yeast
- Prebiotics
- Phytochemicals

Increase gut permeability (water and electrolytes into intestine)

Diarrhea

= ETEC (Enterotoxigenic E. coli)

= Toxins

Colonization and release of toxins

Alternative antimicrobial approaches against ETEC

- Direct-fed microbials
- Oligosaccharides
- Yeast
- Prebiotics
- Phytochemicals
Ingestion of E. coli

Attachment of E. coli to receptors through fimbriae

Colonization and release of toxins

Increase gut permeability (water and electrolytes into intestine)

✔ Fiber
✔ Direct-fed microbials
✔ Zinc oxide

=ETEC (Enterotoxigenic E. coli)
=Toxins
Ingestion of *E. coli*

- Attachment of *E. coli* to receptors through fimbriae
- Colonization and release of toxins
- Increase gut permeability (water and electrolytes into intestine)

- Diarrhea
  - =ETEC (Enterotoxigenic *E. coli*)
  - =Toxins

Alternative antimicrobial approaches against ETEC

- **✓** Oligosaccharides
- **✓** Direct-fed microbials
- **✓** Prebiotics
Blood group antigen oligosaccharides: Receptor for fimbrial subunit FedF

FedF

Key

- Galactose
- N-Acetylglucosamine
- N-Acetylgalactosamine
- Fucose

Type O

Type A

Type B

Source: Moonens et al., 2012
Epsilon-poly-lysine (ε-PL): Stable delivery vehicle

Source: Chen et al, 2021
Grafted polymer: Potential synergistic effects

Type A

$\varepsilon$-PL
To investigate the efficacy of blood group A type-based polymer on intestinal health and disease resistance of weanling pigs challenged with ETEC F18.
**Experimental design & treatments**

- **Experimental design:** RCBD (Blocks: BW x Sex)
- **48 weaning pigs (7.23 ± 1.14 kg BW, 21 d old)**
- **Treatment:** 4 treatments (12 pigs/treatment)

| Treatment                        | Description                                      |
|----------------------------------|--------------------------------------------------|
| Nursery basal diet as control (CON) |                                                   |
| CON + 10 mg/kg of oligosaccharide-based polymer* (LOW) | Glycoconjugate composed of blood group A antigen oligosaccharides grafted on carrier and was designed and synthesized by Elicityl (France) in cooperation with Dr. Eric Cox (Ghent Univ., Belgium) and provided by Pancosma (Geneva, Switzerland) |
| CON + 20 mg/kg of oligosaccharide-based polymer* (HIGH) |                                                   |
| CON + 50 mg/kg of antibiotics (Carbadox; CAR) |                                                   |

*E. coli challenged

*Glycoconjugate composed of blood group A antigen oligosaccharides grafted on carrier and was designed and synthesized by Elicityl (France) in cooperation with Dr. Eric Cox (Ghent Univ., Belgium) and provided by Pancosma (Geneva, Switzerland)*
Experimental timeline & Data acquisition

ETEC F18 challenge (LT, STb, SLT-2); oral inoculation, $10^{10}$ cfu/dose per 3 mL in PBS

- Growth performance
- Diarrhea severity
- β-hemolytic coliforms
- Bacterial translocation
- Intestinal morphology
- Gene expression in intestinal mucosa

* PI=post-inoculation
PBS= phosphate-buffered saline
OBP supplementation enhanced feed efficiency and reduced diarrhea

**Gain:Feed**

| PI | CON | LOW | HIGH | CAR |
|----|-----|-----|------|-----|
| -7 to 0 | b | ab | b | a |
| 0 to 5 PI | a | a | a | a |
| 5 to 11 PI | b | ab | a | ab |

**Frequency of diarrhea**

| PI | CON | LOW | HIGH | CAR |
|----|-----|-----|------|-----|
| d 0 to 11 PI | a | b | b | b |

Plots show the frequency of diarrhea over different periods post-inoculation (PI) with supplementation levels: CON, LOW, HIGH, CAR. Each bar represents a different period and is labeled with a or b to indicate statistical significance.

PI=post-inoculation

UC DAVIS
OBP supplementation enhanced ETEC excretion, thus reduced bacterial translocation.

**β-hemolytic coliforms in feces**

|                | CON | LOW | HIGH | CAR |
|----------------|-----|-----|------|-----|
| d 2 PI (%)     | a a a | a a a | a a a | a a a |
| d 5 PI (%)     | a a a | a a a | b b b | a a a |
| d 8 PI (%)     | a ab ab b | a ab ab b | a ab ab b | a ab ab b |
| d 11 PI (%)    | a a b | a a b | a a b | a a b |

**Bacterial translocation**

|                | CON | LOW | HIGH | CAR |
|----------------|-----|-----|------|-----|
| Mesenteric lymph node cfu/g | a a | a a | a b | a b |
| Spleen cfu/g | b b | b b | b b | b b |

d = post-inoculation
OBP supplementation is beneficial for pigs’ intestinal morphology

Villous height, d 5 PI

Villous height, d 11 PI

Pl = post-inoculation
OBP supplementation is beneficial for pigs’ intestinal health

Gene expression profiles in jejunal mucosa, d 5 PI

| Gene   | CON | LOW | HIGH | CAR |
|--------|-----|-----|------|-----|
| MUC2   |     |     | a    |     |
| CLDN1  |     | b   | a    |     |
| ZO1    | ab  |     | a    | ab  |
| OCDN   |     |     | a    |     |

Gene expression profiles in ileal mucosa, d 5 PI

| Gene   | CON | LOW | HIGH | CAR |
|--------|-----|-----|------|-----|
| IL1B   |     | ab  | a    |     |
| IL6    |     | b   | a    | ab  |
| TNF    |     | ab  | a    |     |
| PTGS2  |     |     |     |     |

PI=post-inoculation
Key takeaways

Oligosaccharide-based polymer supplement enhanced disease resistance of weaned pigs

Smart use of antibiotics:
Minimize the use of antibiotics and explore the possible alternatives

The global food crisis:
Developing sustainable livestock production system
Supplementation of oligosaccharide-based polymer enhanced growth and disease resistance of weaned pigs by modulating intestinal integrity and systemic immunity

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Thank you for your attention!
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