

Young Scholar Presentation

Dietary supplementation of *Bacillus subtilis* influenced intestinal health and metabolomic profiles of weaned pigs experimentally infected with a pathogenic *E. coli*

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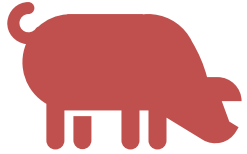
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Outline



Impacts of weaning stress on intestinal development and health of pigs



How to define a healthy gut



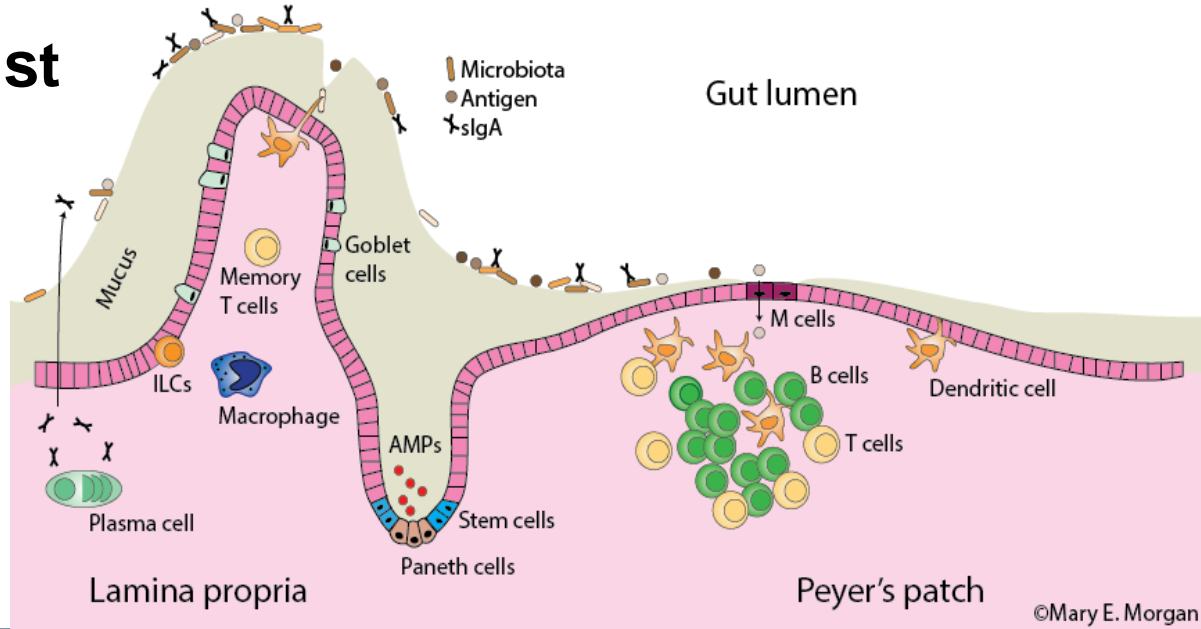
Research objective & methods



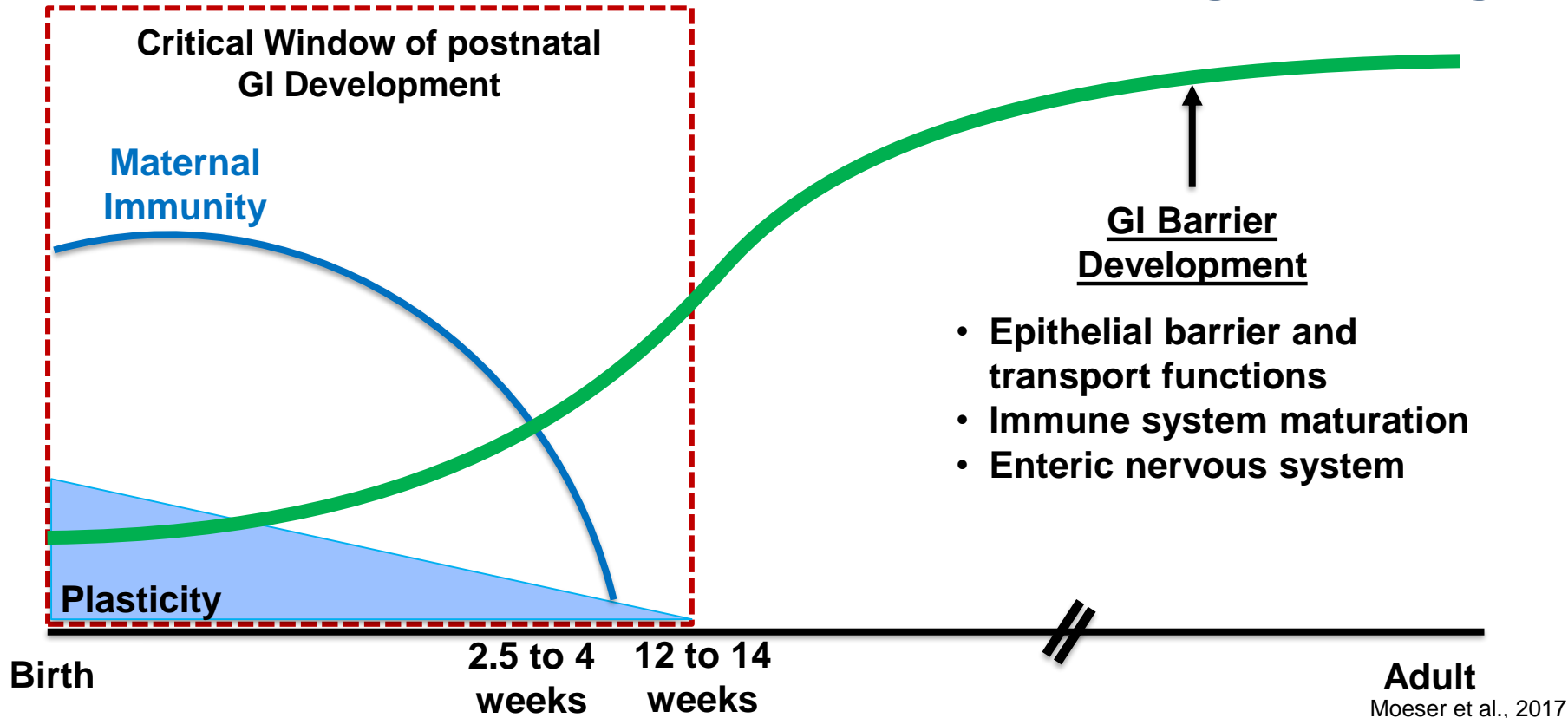
Results & conclusions

Importance of the gastrointestinal (GI) tract development

- Digestion and absorption
- Physical barrier against pathogens
- Largest part of the immune system



Gastrointestinal (GI) tract development during weanling

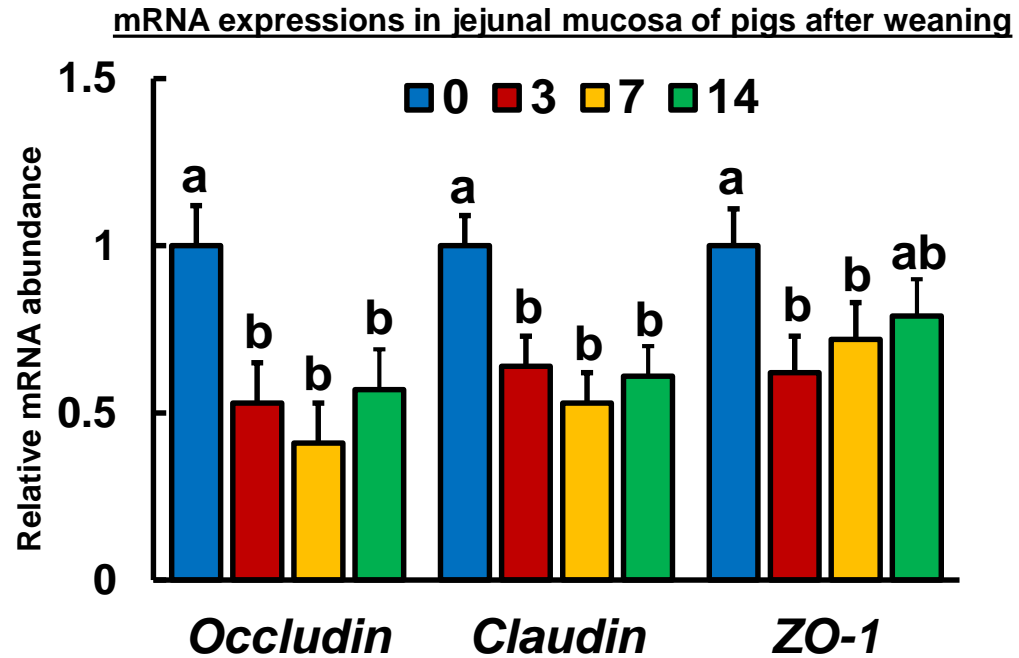
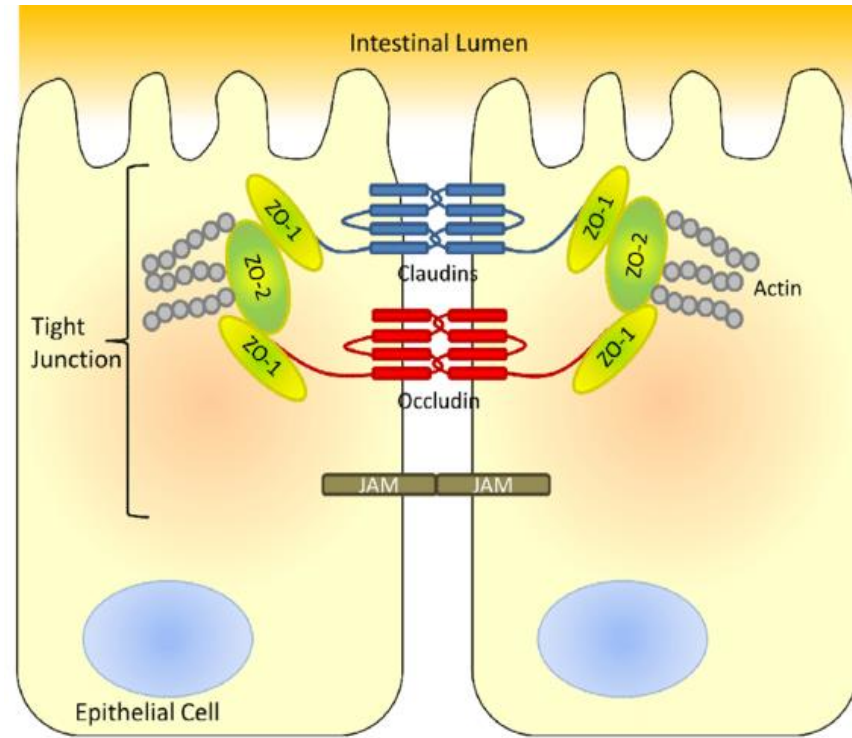


Weaning stress

- Environmental changes
- Transportation stress
- Abrupt transition of diet
- Increased exposure to pathogens

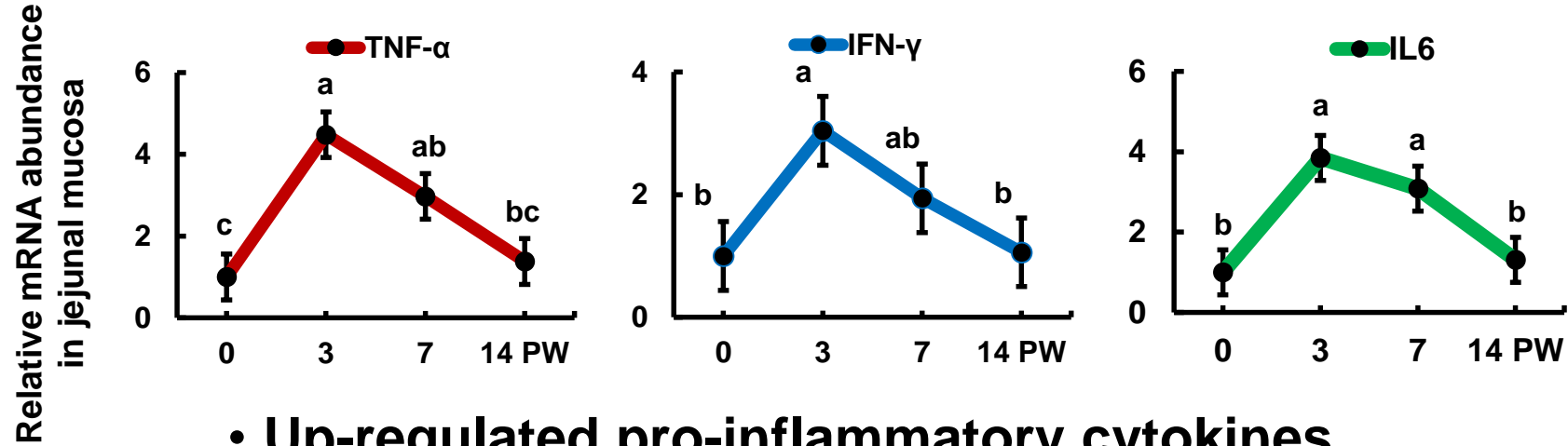


Weaning stress on intestinal barrier function



Hu et al., 2013

Weaning stress on intestinal mucosal immunity



- Up-regulated pro-inflammatory cytokines
- Increased CD4+ and CD8+ T lymphocytes
- Reduced secretory IgA

*PW=post-weaning

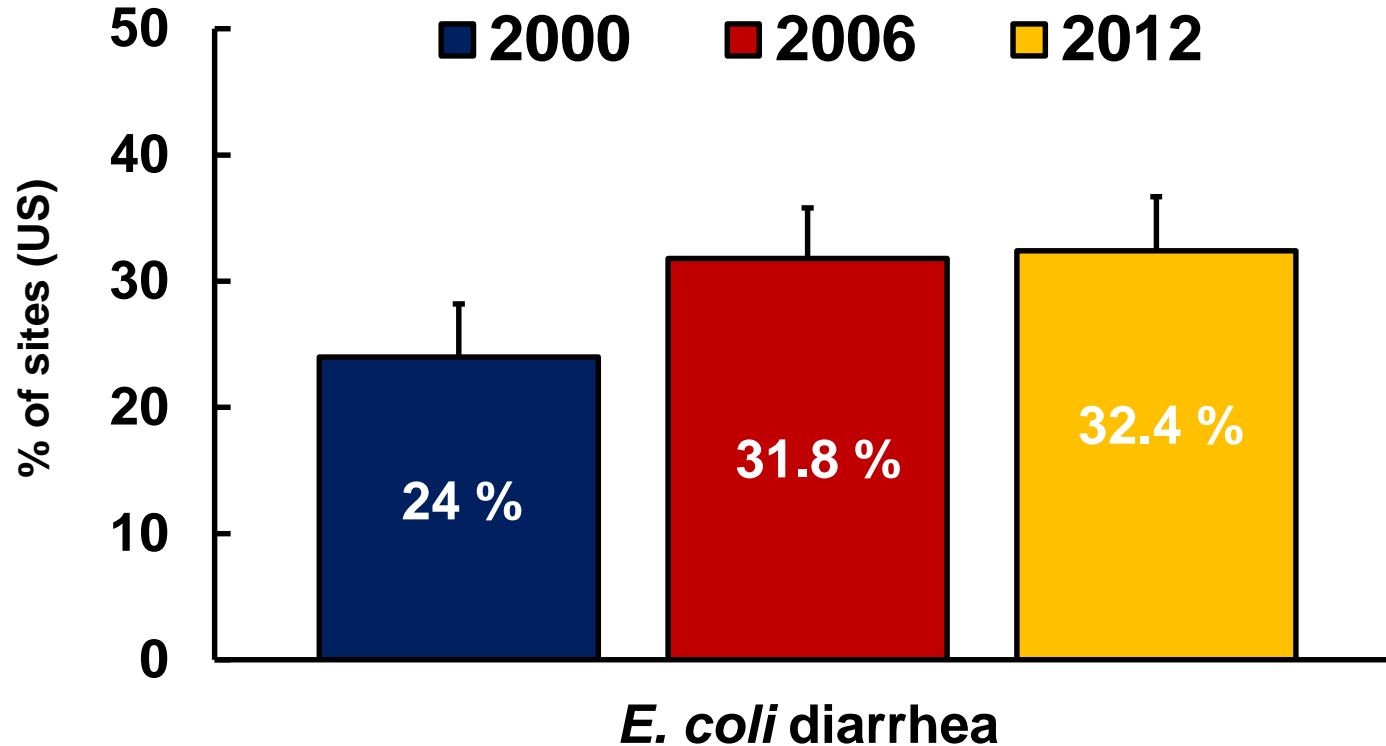
Hu et al., 2013

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

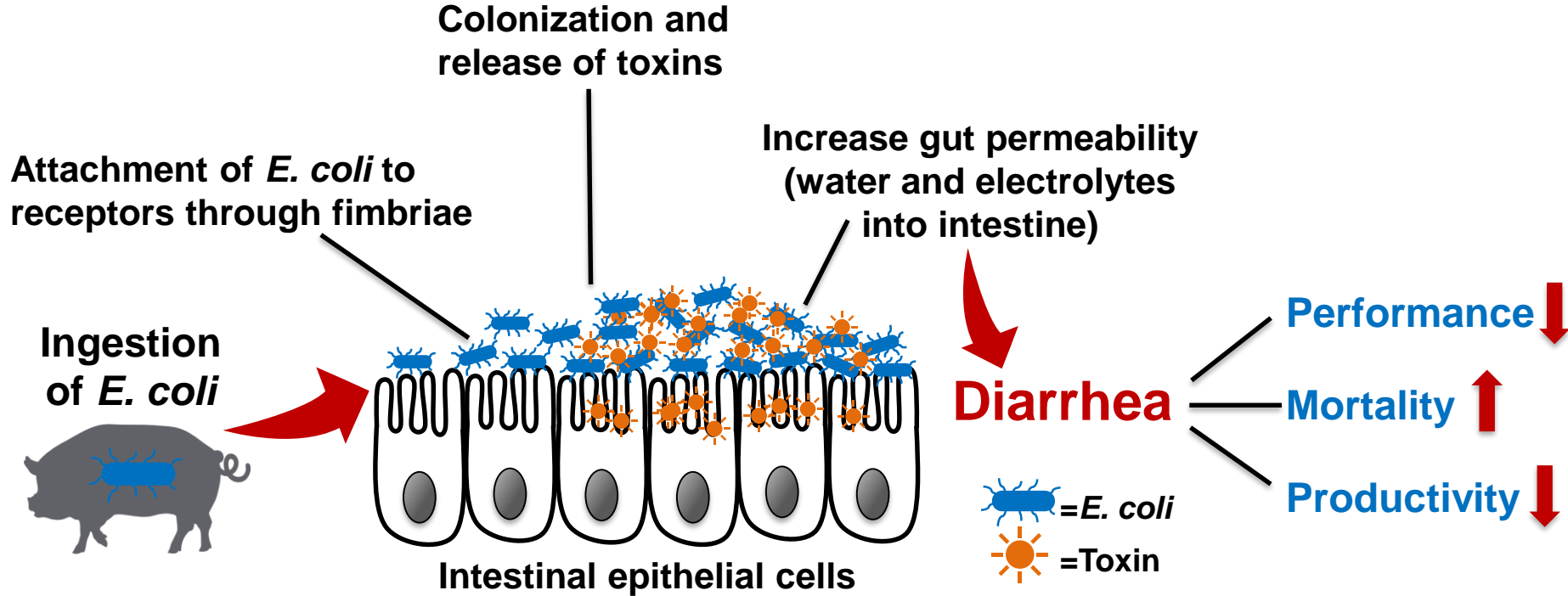


Post-weaning *E. coli* diarrhea morbidity



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

Post-weaning *E. coli* diarrhea



Toxins in *E. coli*

- **Heat-labile toxin (LT)**

- ✓ Stimulating secretion of water/ions (K^+ and Cl^-)

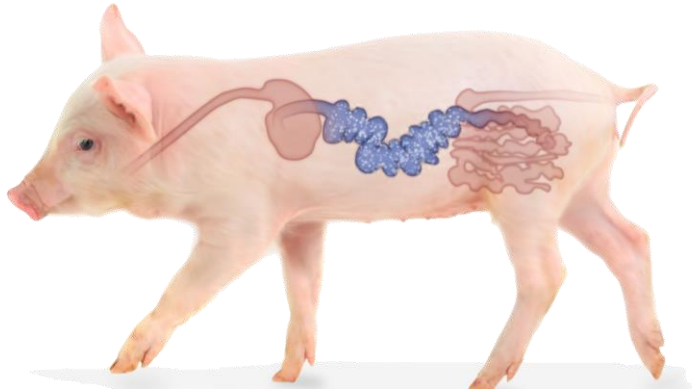
- **Heat-stable toxin (ST)**

- ✓ Inhibiting absorption of water/ions (Na^+ and Cl^-)
- ✓ Histological damage

- **Other toxins (hemolysin, shiga toxin)**

- ✓ Hemolysis

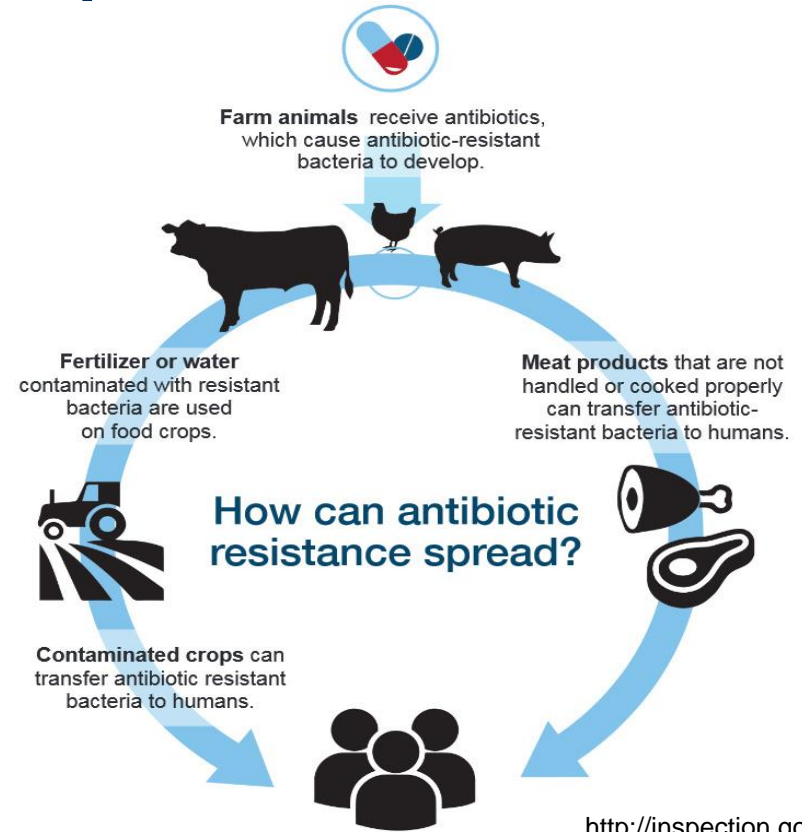
How to define a healthy gut?



Pluske et al., 2018

Antibiotic resistance as a major public health concern

- **Banned antibiotics as growth promoter in the E.U and U.S since 2006, and 2017, respectively**
- **Alternatives to antibiotic are highly demanded**



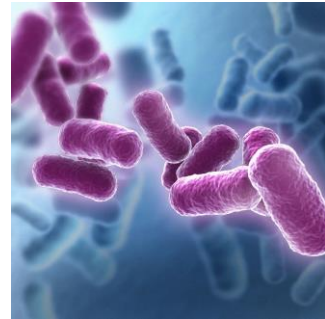
<http://inspection.gc.ca>

Direct-fed microbials (DFM)

- DFM, also known as probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2001)
- DFMs are non-nutrient feed additives and potential alternatives to antibiotics
 - ✓ *Bacillus spp.* (Gram +, spore-formers)
 - ✓ **Lactic acid-producing bacteria**
 - *Lactobacillus spp.*, *Bifidobacterium spp.*, *Enterococcus spp.*
 - ✓ **Yeast and fungi**

***Bacillus*-based DFM**

- High resistance of its spores to harsh environment and more stable at ambient temperature
- *Bacillus*- based DFM have been identified as potent producers of extracellular fiber-degrading enzymes, which may aid nutrient digestion and utilization
(Ferrari et al., 1993; Schreier, 1993)



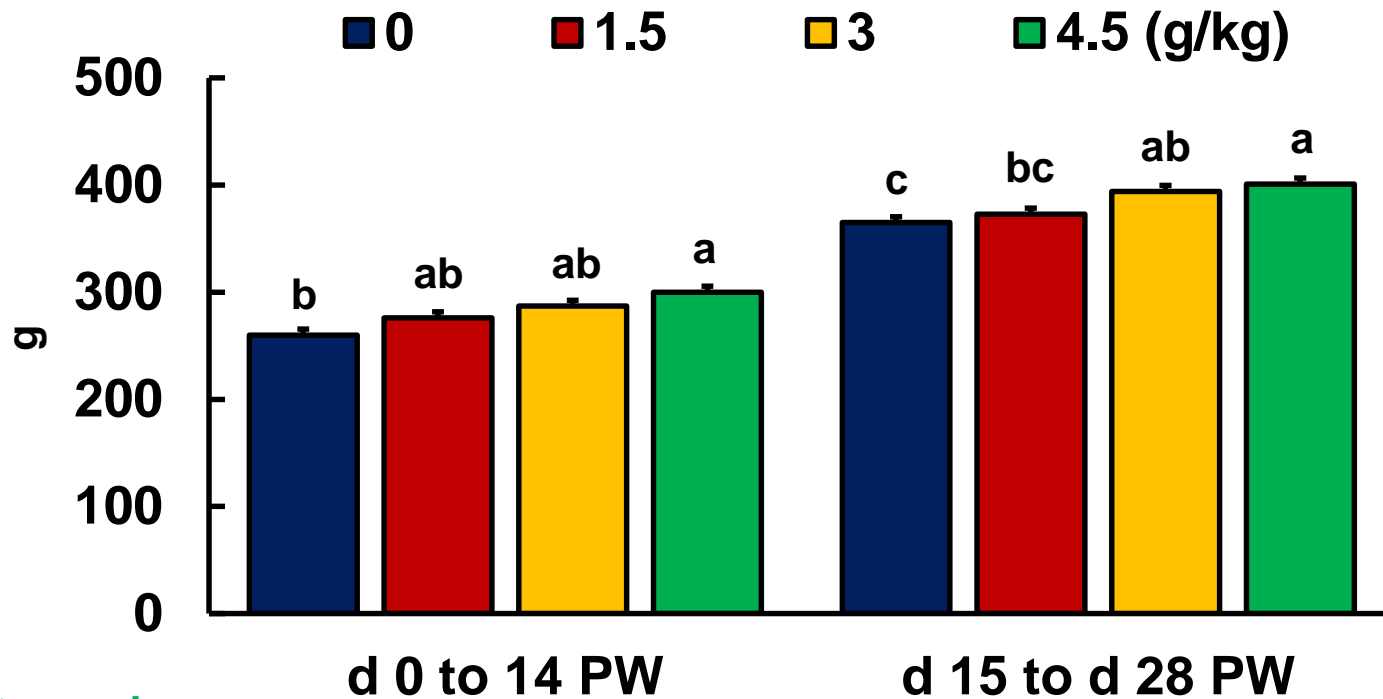
Bacillus subtilis

- **Gram-positive endospore-forming bacteria (member of the genus *Bacillus*)**
- **Well studied of bacterial chromosome replication and cell differentiation**



<https://wickhamlabs.co.uk/technical-resource-centre>

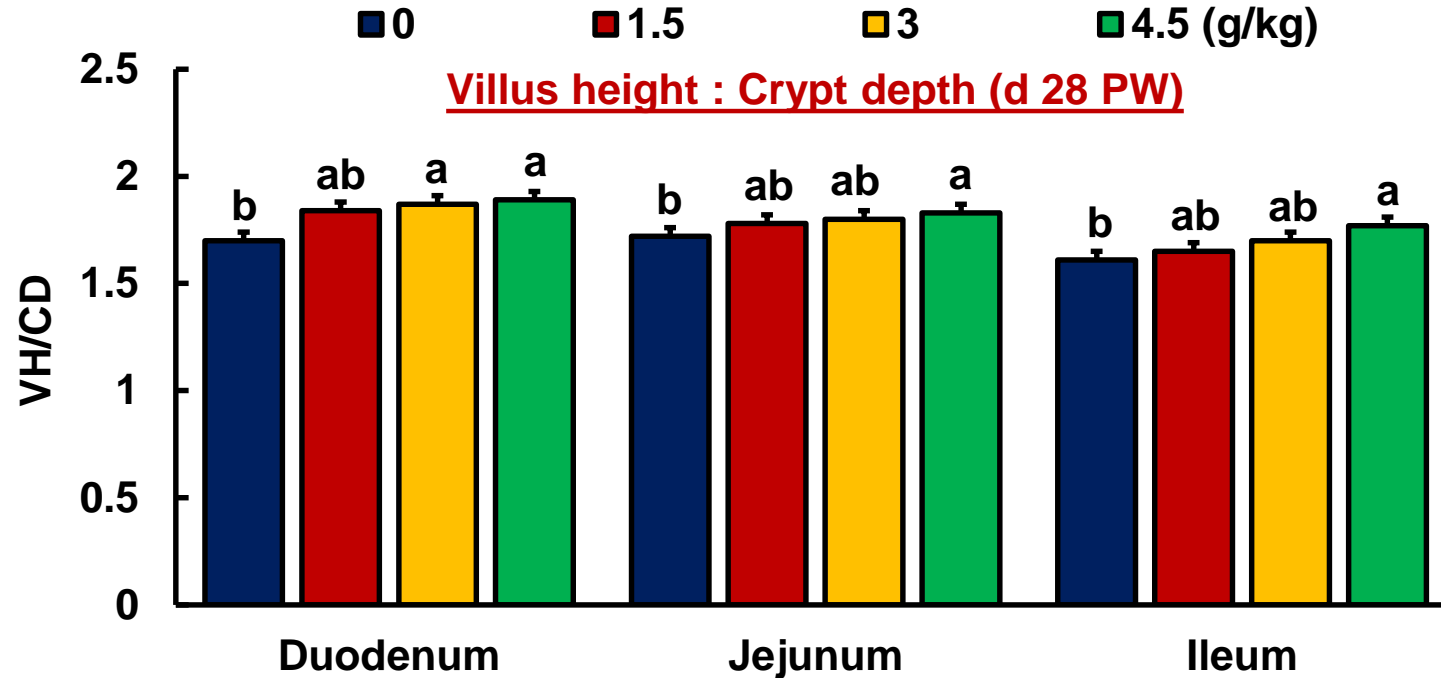
Bacillus subtilis improved average daily gain



*PW=post-weaning

Lee et al., 2014

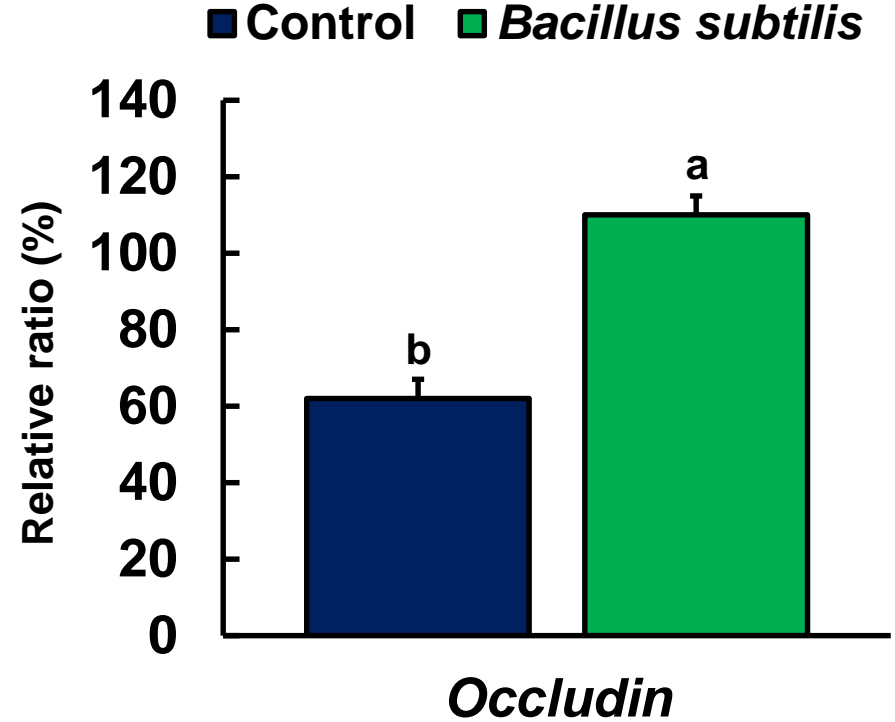
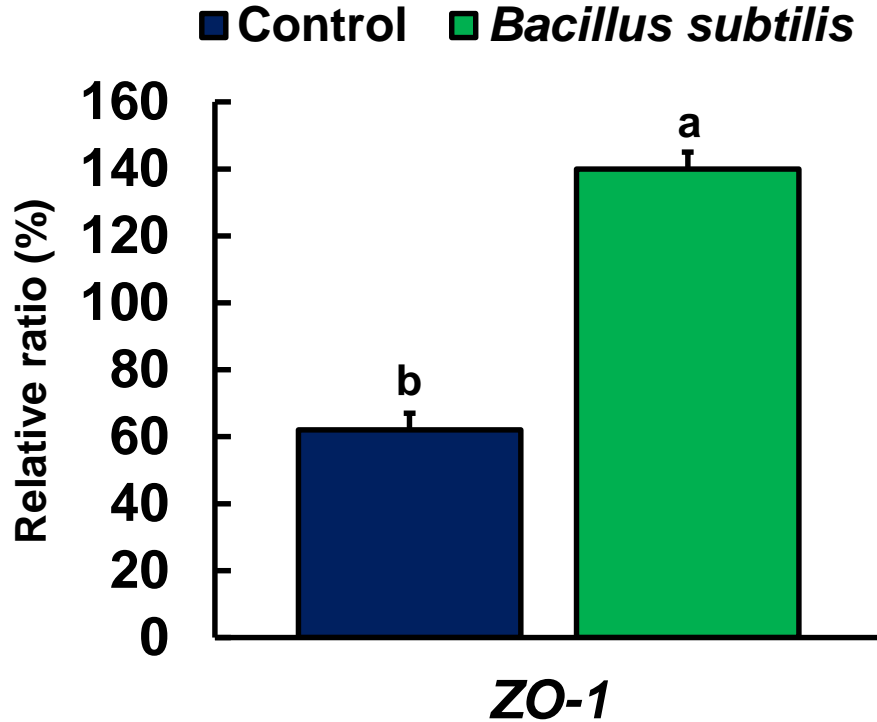
Bacillus subtilis enhanced intestinal morphology



*PW=post-weaning

Lee et al., 2014

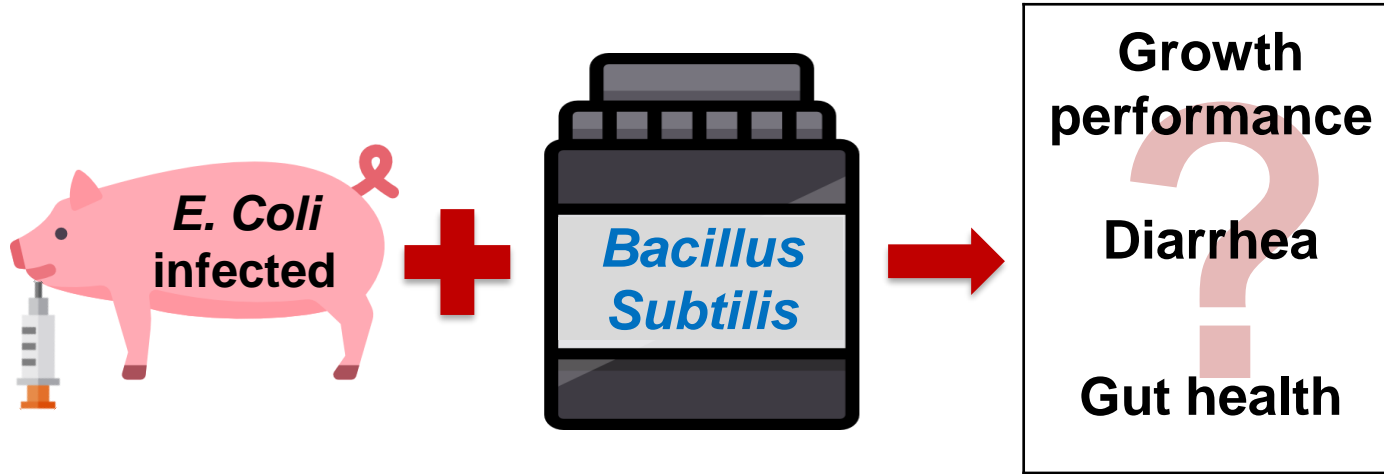
Bacillus subtilis up-regulated tight junction proteins



Experiment conducted using porcine intestinal epithelial cell line (IPEC-J2)

Gu et al., 2014

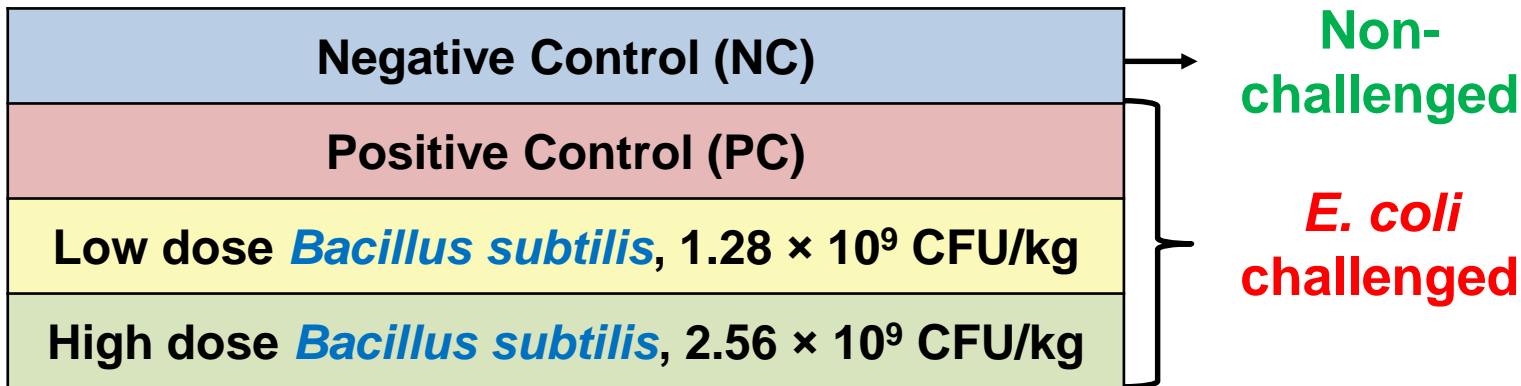
Research objective



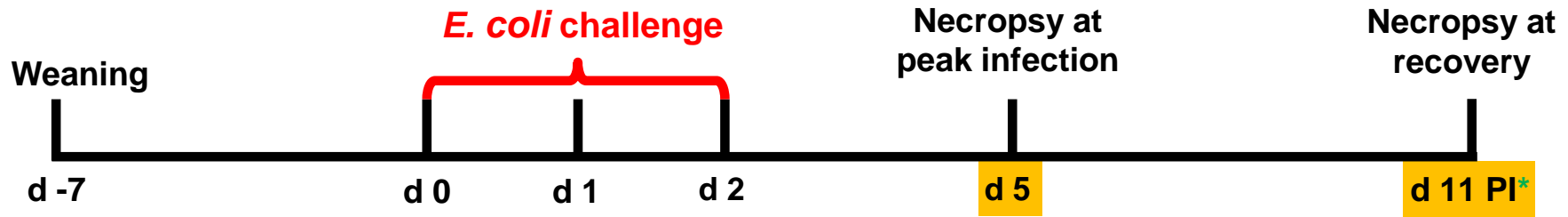
Investigate dietary supplementation of *Bacillus subtilis* (DSM 25841) on growth performance, intestinal health, metabolomic profiles and gut microbiota of weaned pigs experimentally infected with a pathogenic F18 *E. coli*.

Experimental design & treatments

- Experimental design: RCBD (Blocks: BW x Sex)
- 48 weaning pigs (6.73 ± 0.77 kg BW, 21 d old)
- Treatment: 4 treatments (12 pigs/treatment)



Experimental timeline & data collection



➤ Pathogenic F18 *E. coli* challenge (LT, STb, SLT-2); oral inoculation, 10^{10} cfu/dose with 3 doses

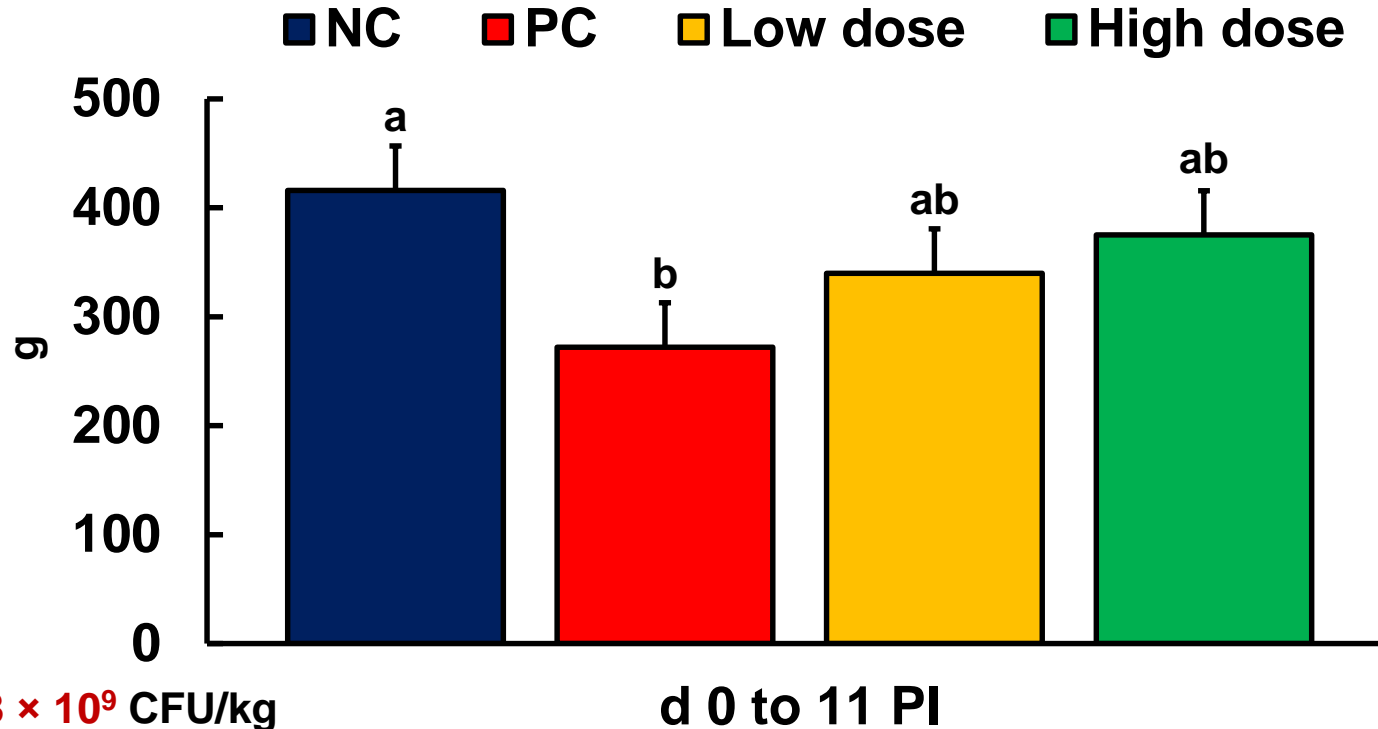
- Growth performance
- Daily diarrhea score
- Gut permeability of jejunum with Ussing Chamber
 - ✓ Transcellular (Horseradish peroxidase)
 - ✓ Paracellular (FITC-4000)

*PI=post-inoculation

Data collection cont.

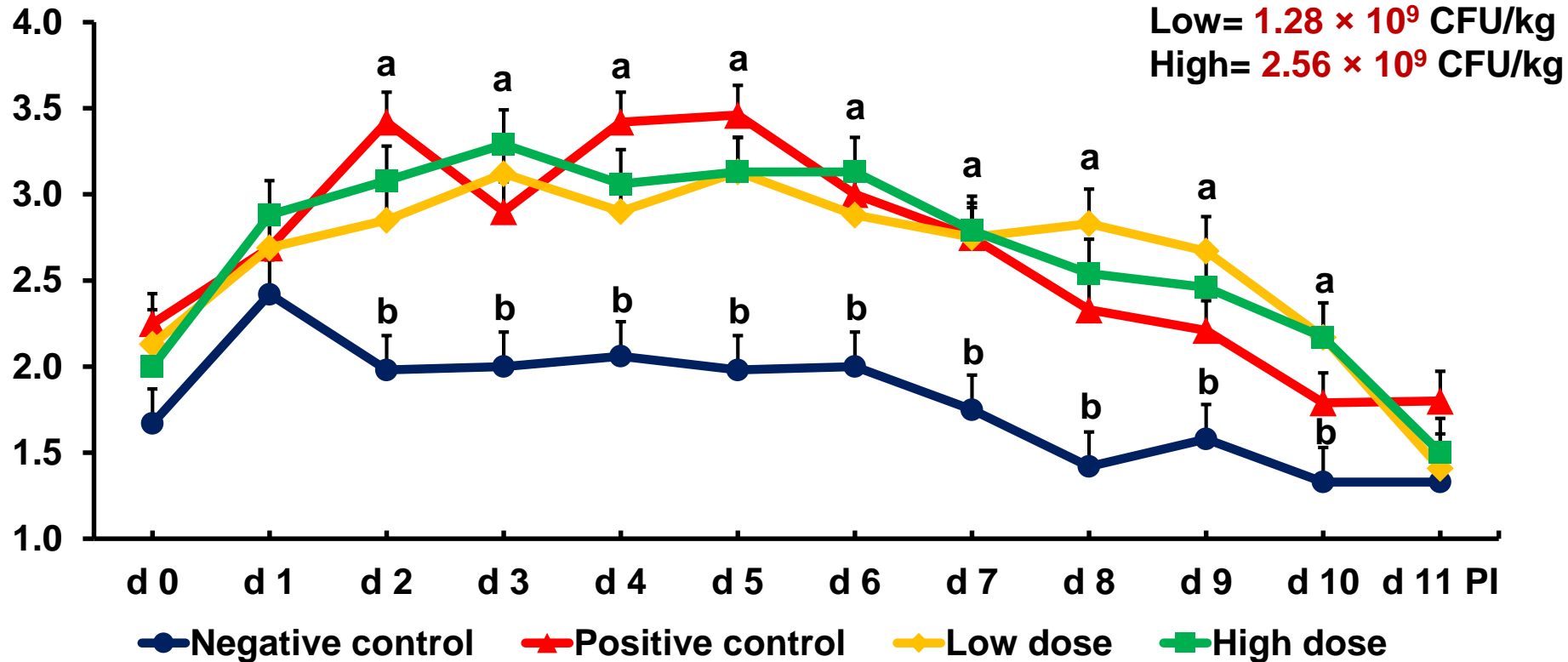
- **Gene expression in intestinal mucosa by qPCR**
 - ✓ Jejunal mucosa (gut barrier function and nutrient transport)
 - ✓ Ileal mucosa (immune defense)
- **Colon digesta metabolomic profiles by gas chromatography time of flight-mass spectrometer (GCTOF-MS)**
 - ✓ All processed data were analyzed by MetaboAnalyst (<http://www.metaboanalyst.ca>)
 - ✓ Fold change > 1.5; FDR < 0.2; $P < 0.05$
- **Colon microbiota was analyzed using 16S rRNA gene sequencing and compositional data was analyzed using QIIME2 (2016. 6).**
- **All data were analyzed by ANOVA using the PROC MIXED of SAS**

Average daily gain

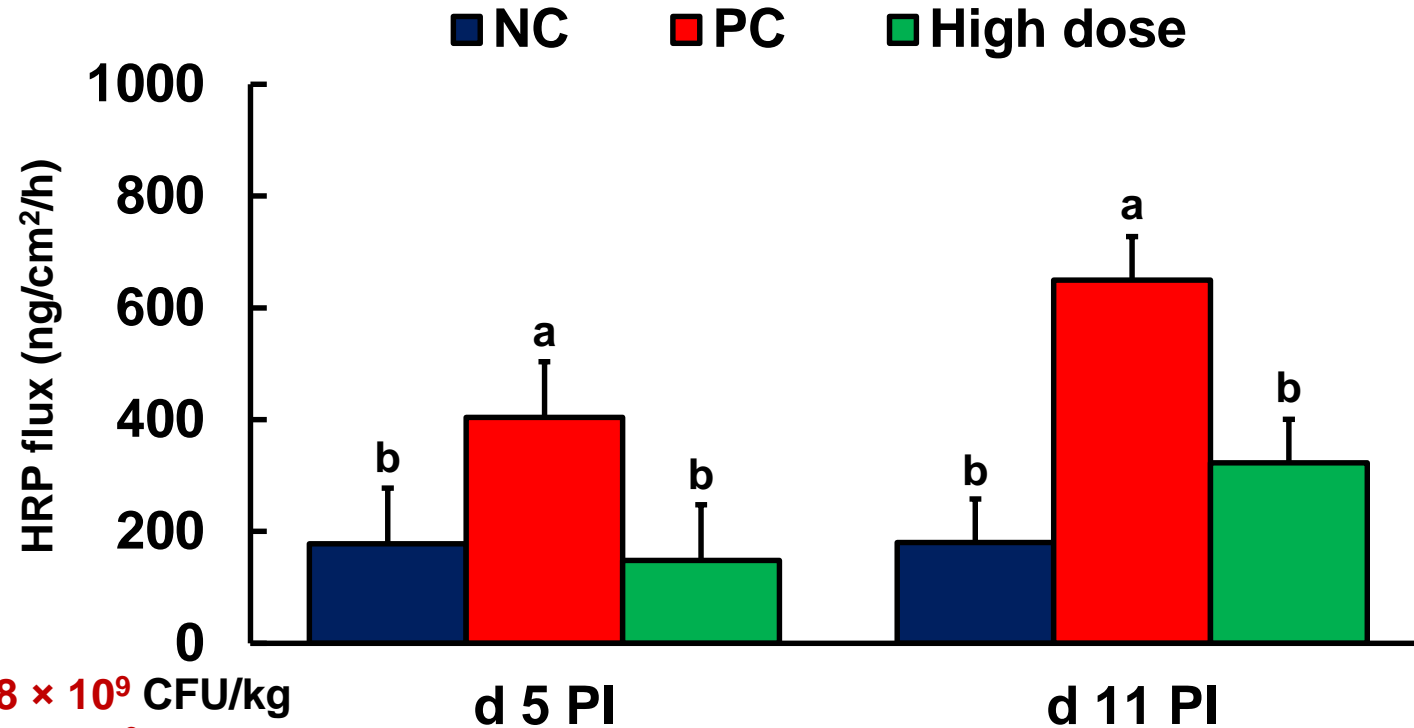


Low= 1.28×10^9 CFU/kg
High= 2.56×10^9 CFU/kg

Daily diarrhea score

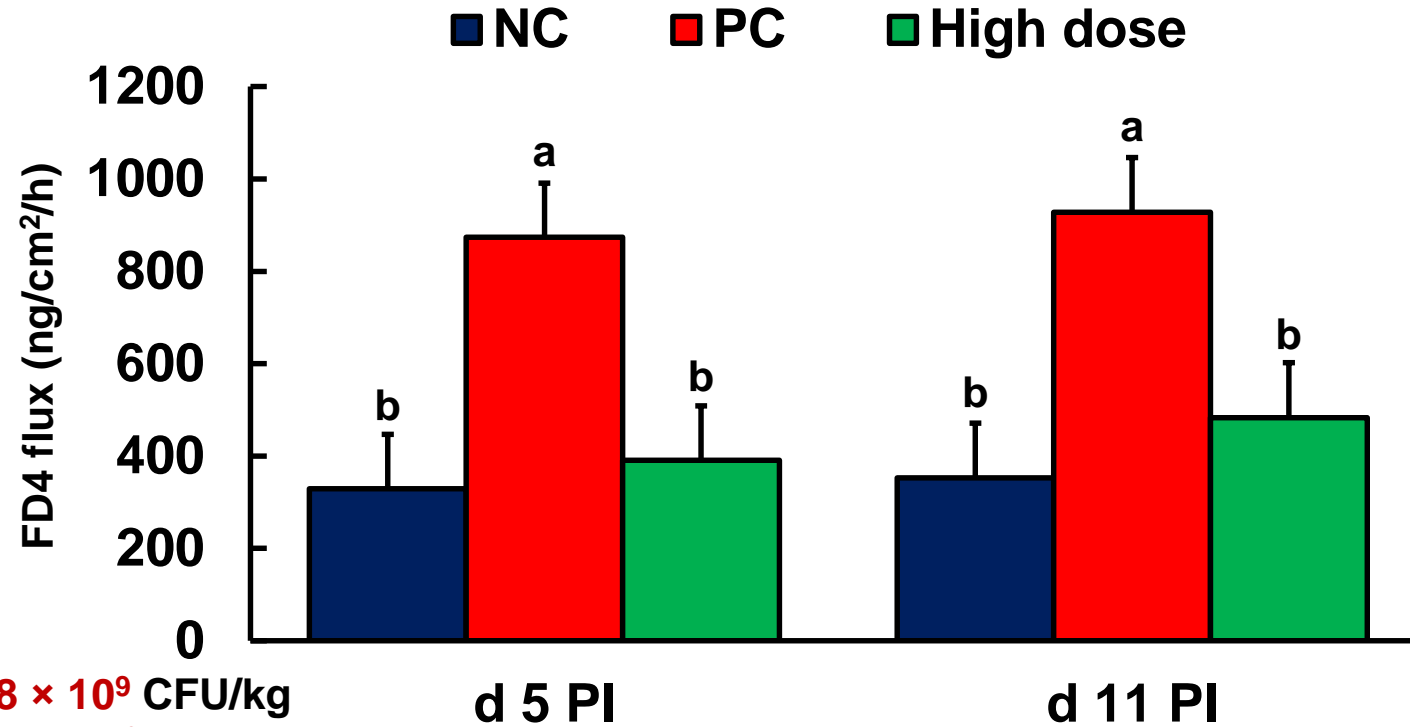


Transcellular permeability of jejunum



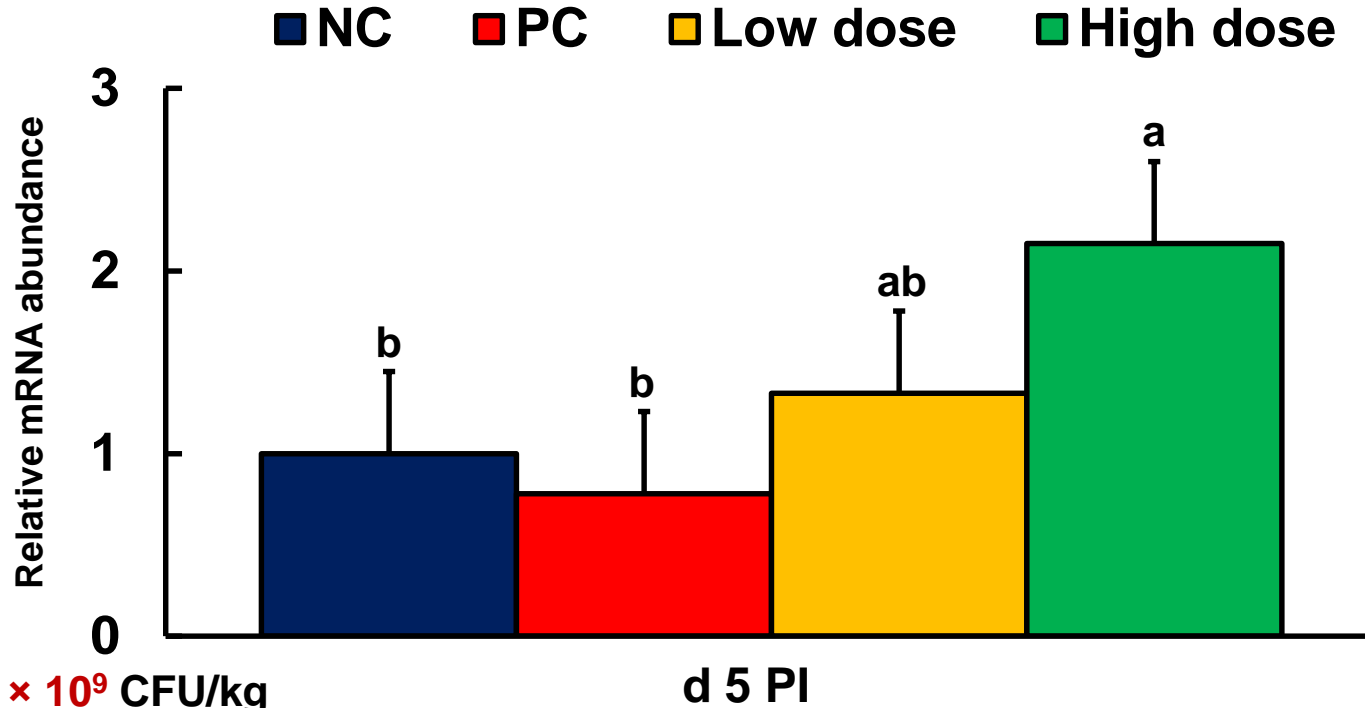
Low= 1.28×10^9 CFU/kg
High= 2.56×10^9 CFU/kg

Paracellular permeability of jejunum



Low= 1.28×10^9 CFU/kg
High= 2.56×10^9 CFU/kg

ZO1 expression in jejunal mucosa



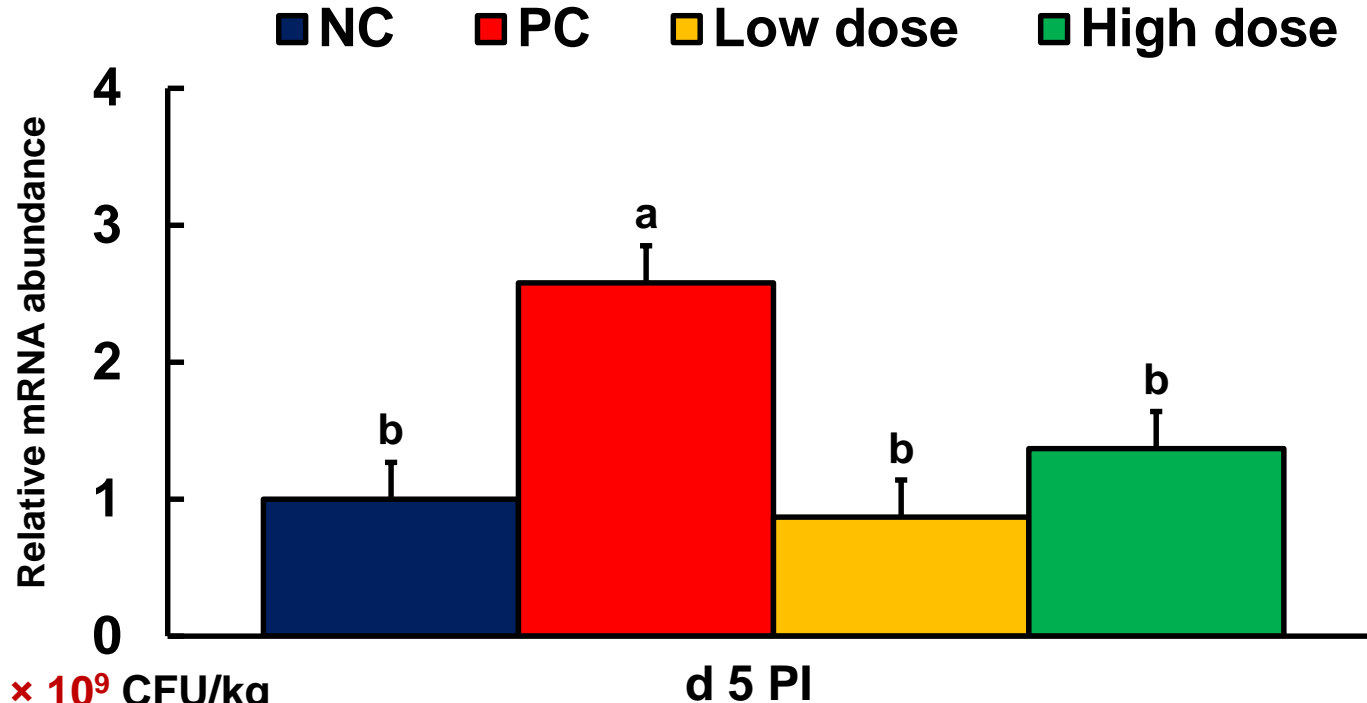
Low= 1.28×10^9 CFU/kg

High= 2.56×10^9 CFU/kg

d 5 PI

No differences were observed in occludin and claudin

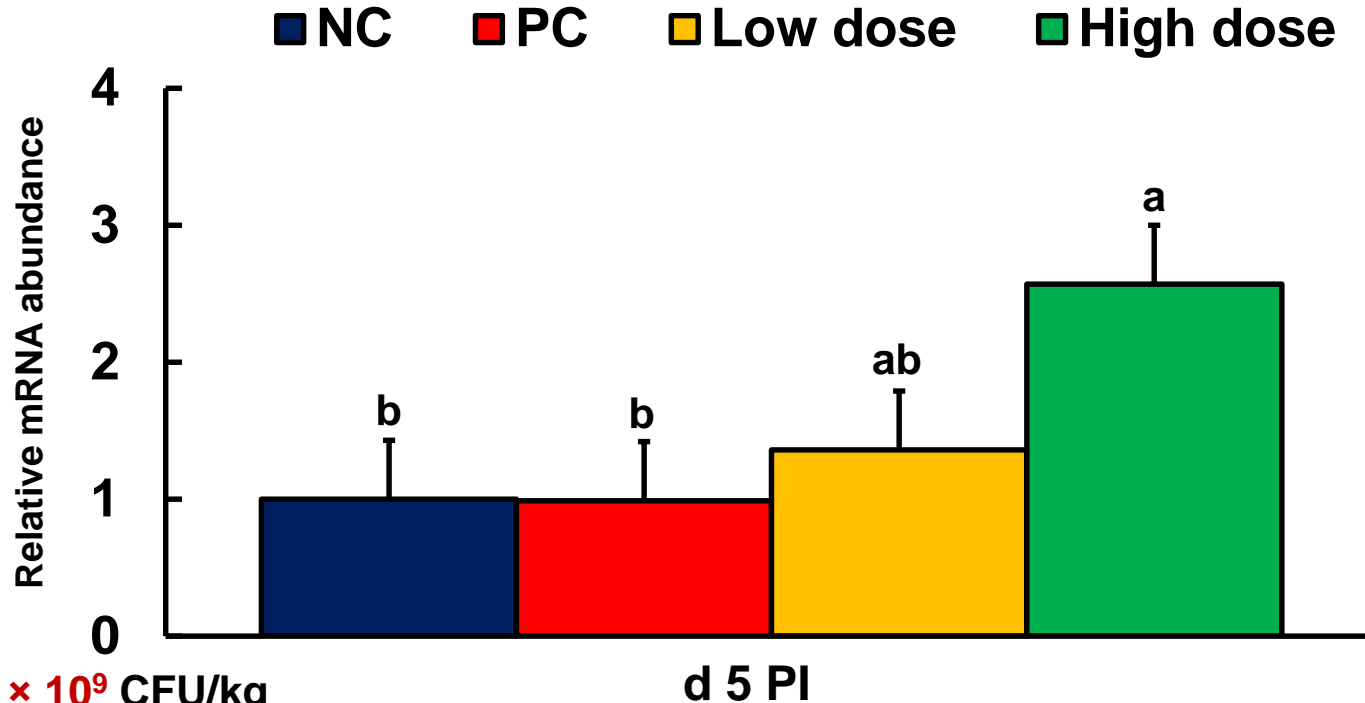
MUC2 expression in jejunal mucosa



Low= 1.28×10^9 CFU/kg

High= 2.56×10^9 CFU/kg

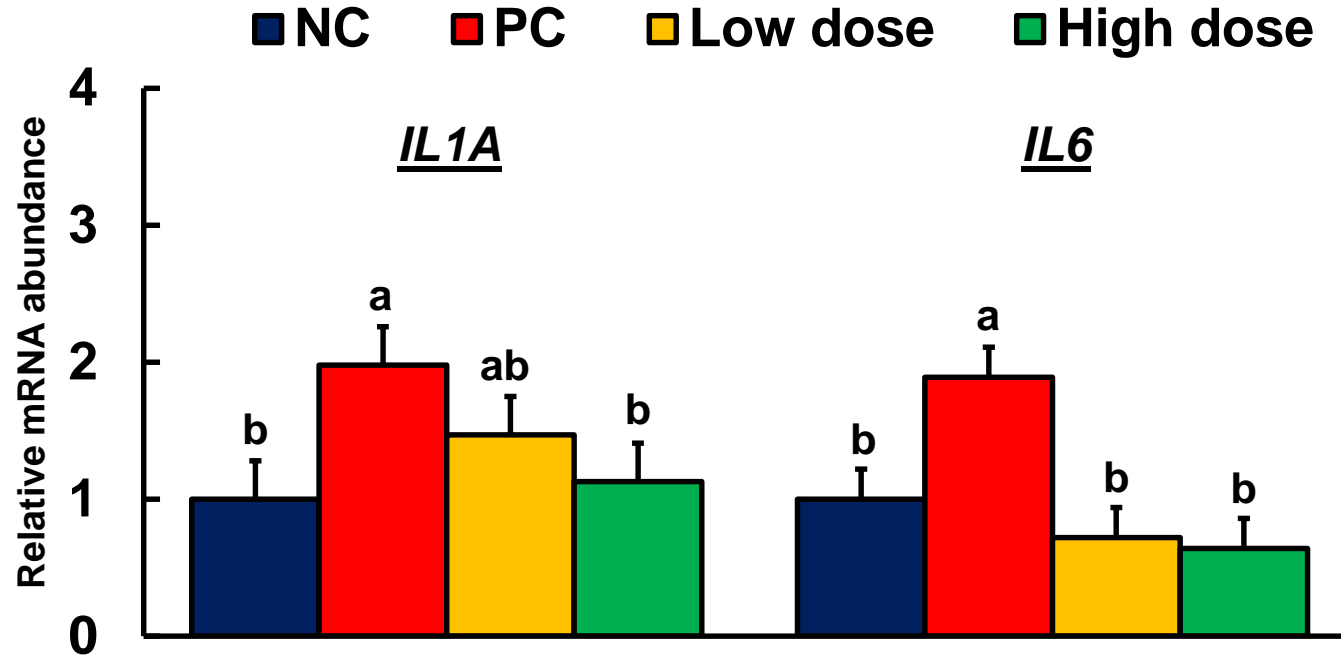
CFTR expression in jejunal mucosa



Low= 1.28×10^9 CFU/kg

High= 2.56×10^9 CFU/kg

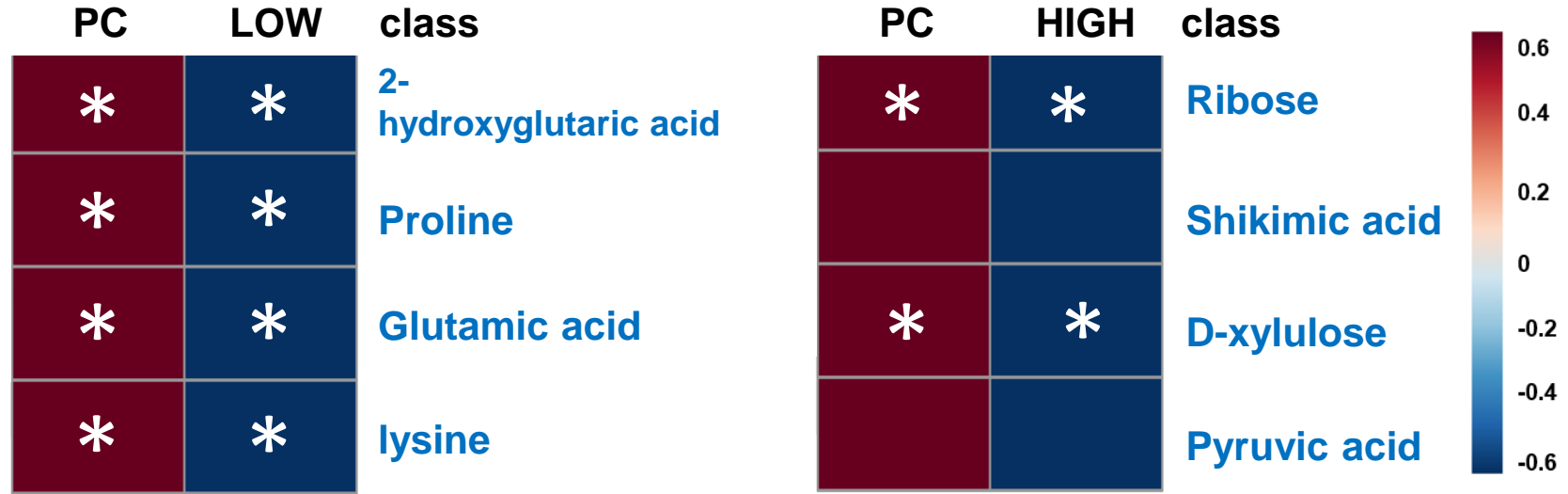
Pro-inflammatory cytokines gene expression in ileal mucosa



Low= 1.28×10^9 CFU/kg

High= 2.56×10^9 CFU/kg

Distal colon metabolomic profiles: Heatmap and hierarchical clustering, d 5 PI



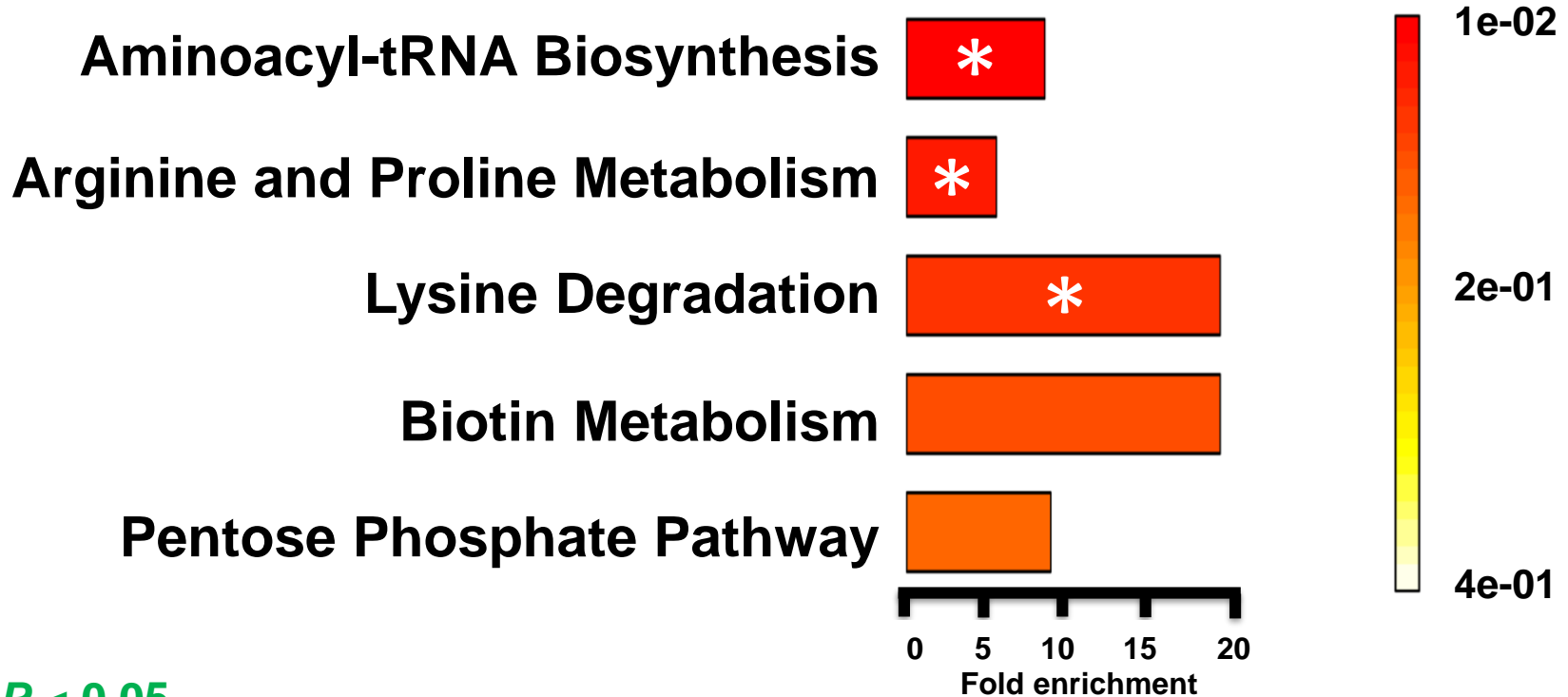
* Fold change > 1.5; FDR < 0.2

Low= 1.28×10^9 CFU/kg

High= 2.56×10^9 CFU/kg

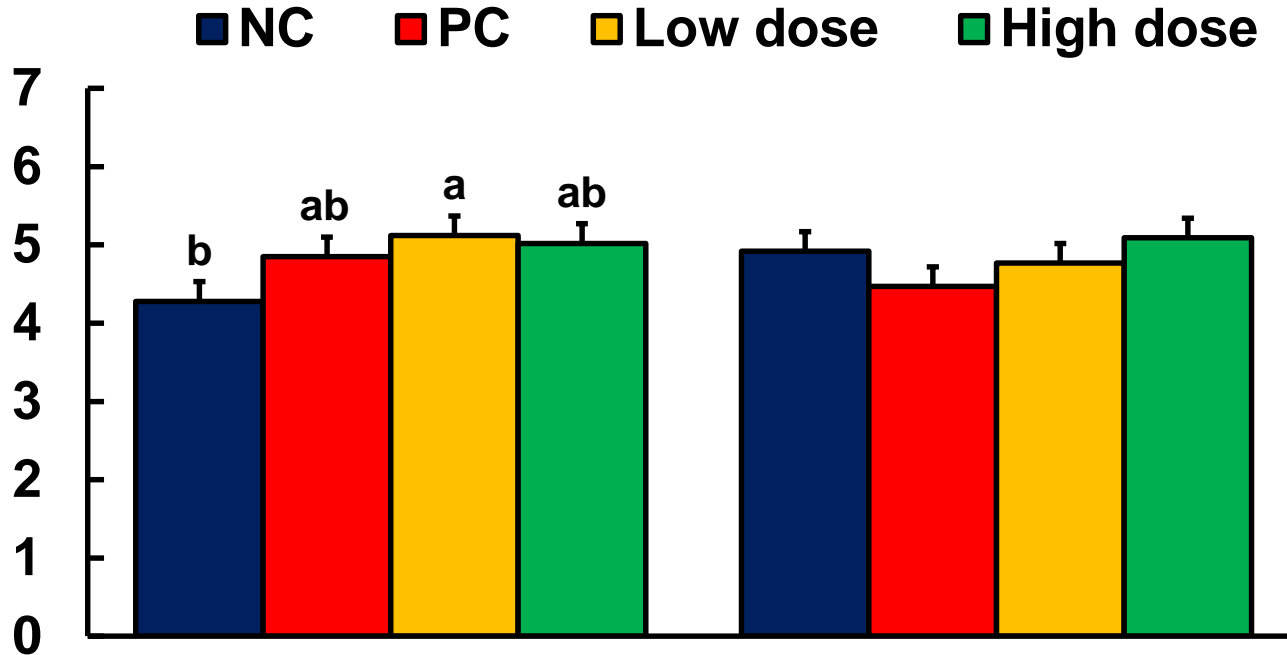
No differences were observed in NC vs PC on d 5 PI;
No differences were observed in d 11 PI

Distal colon metabolomic profiles: Metabolite sets enrichment, d 5 PI



* $P < 0.05$

Alpha diversity: Shannon



Low= 1.28×10^9 CFU/kg

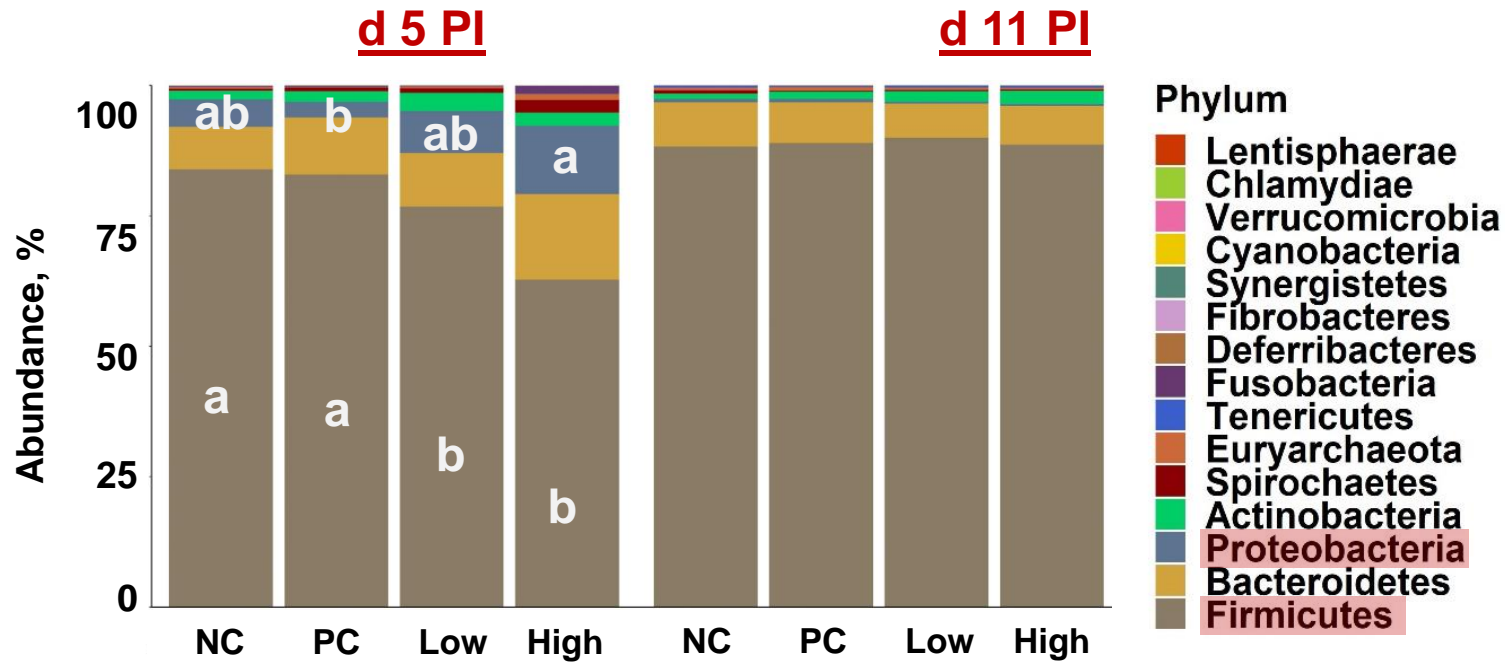
d 5 PI

d 11 PI

High= 2.56×10^9 CFU/kg

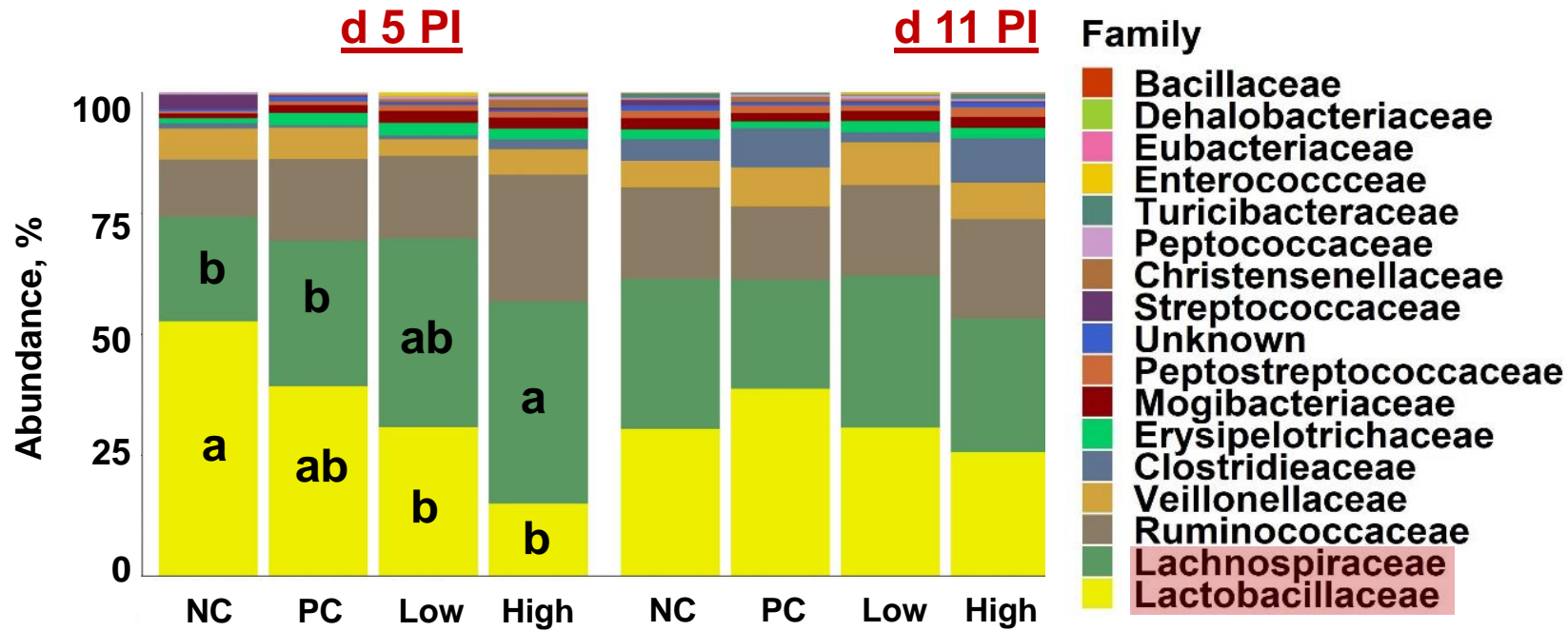
Relative abundance: Phylum

- ✓ Lowest relative abundance of *Proteobacteria* in PC
- ✓ Lowest relative abundance of *Firmicutes* in *Bacillus subtilis*



Relative abundance: Firmicutes

- ✓ Highest relative abundance of *Lachnospiraceae* in High dose
- ✓ Lowest relative abundance of *Lactobacillaceae* in *Bacillus subtilis*



Conclusions

Supplementation of *Bacillus subtilis* (DSM 25841)

- Enhanced the growth rate
 - Reduced gut permeability
 - Enhanced gut barrier functions
 - Reduced intestinal inflammation
 - Modified metabolomic profiles and gut microbiota
- of weaned pigs experimentally infected with F18 *E. coli*.

Acknowledgement



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RESEARCH

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Thank you for your attention!

