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Antibacterial plant blends modulate gut microbiota in organic piglets challenged with *E. coli* F18

K. Jerez-Bogota[1,2], M. Jensen[1], O. Højberg[2] and N. Canibe[2]

[1]DEPARTMENT OF FOOD SCIENCE
[2]DEPARTMENT OF ANIMAL AND VETERINARY SCIENCE

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

Abstract

ANTIBACTERIAL PLANT BLENDS MODULATE GUT MICROBIOTA IN ORGANIC PIGLETS CHALLENGED WITH *E. COLI* F18

Kevin Jerez-Bogota[1,2], Martin Jensen[1], Ole Højberg[2] and Nuria Canibe[2]

[1]Department of Food Science, Aarhus University, 8200 Aarhus N, Denmark

[2]Department of Animal and Veterinary Sciences, Aarhus University, 8830 Tjele, Denmark

The study investigated the use of combinations of garlic and apple pomace, or blackcurrant as potential in-feed alternatives to antibiotics and zinc oxide in combating postweaning diarrhea caused by enterotoxigenic *E. coli* (ETEC) in organically raised piglets. These blends had previously demonstrated in vitro synergistic antibacterial activity against ETEC. Here we present the effects on ETEC shedding, diarrhea incidence, gut microbiota composition (16S rRNA), and oxidative stress markers. For 21 days, 32 piglets (7-weeks old) were randomly assigned to one of four groups: non-challenge (NC); ETEC-challenged (PC); ETEC-challenged receiving garlic and apple pomace (3%+3%; GA); ETEC-challenged receiving garlic and blackcurrant (3%+3%; GB). A strain of ETEC F18 was administered (8ml; 10^9 CFU/ml) on days 1 and 2 after weaning. Fecal samples were collected daily the first week, and every other day thereafter for diarrhea assessment; microbiota composition was analyzed on days 1, 3, 7, 14 and 21. At the end of the experiment, digesta and mucosa samples from the gastrointestinal tract (GIT) were collected. NC pigs had no ETEC F18 shedding nor diarrheal symptoms. PC group had diarrhea and ETEC shedding, which were reduced in the GA and GB groups. On day 7, the GA, GB, and NC pigs had a greater ($P < 0.05$) Shannon and I-Simpson α-diversity index than the PC pigs. The PC group had a greater ($P < 0.05$) microbiota volatility (compositional change) than all other groups on days 7 and 14. The *Escherichia*, *Campylobacter*, and *Erysipelothrix* genera were less abundant in the NC, GA, and GB than in the PC group (log2FC > 2; $P < 0.05$), whereas *Catenibacterium*, *Dialister*, and *Mitsoukella* were more abundant (log2FC > 2; $P < 0.05$). The GB group had the highest abundance of *Prevotella* and *Lactobacillus* (log2FC > 2, $P < 0.05$). Overall, adding GA or GB to the weaning feed of organic piglets maintained a healthier gut microbiota profile and overall gut health, that reduced the incidence of diarrhea. Results on microbiota along GIT and gut mucosa oxidative stress markers will be further presented.

The study received funding from the EU H-2020 program (grant N° 955374).

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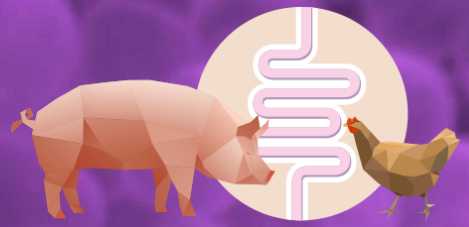

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Introduction

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E. coli and postweaning diarrhea

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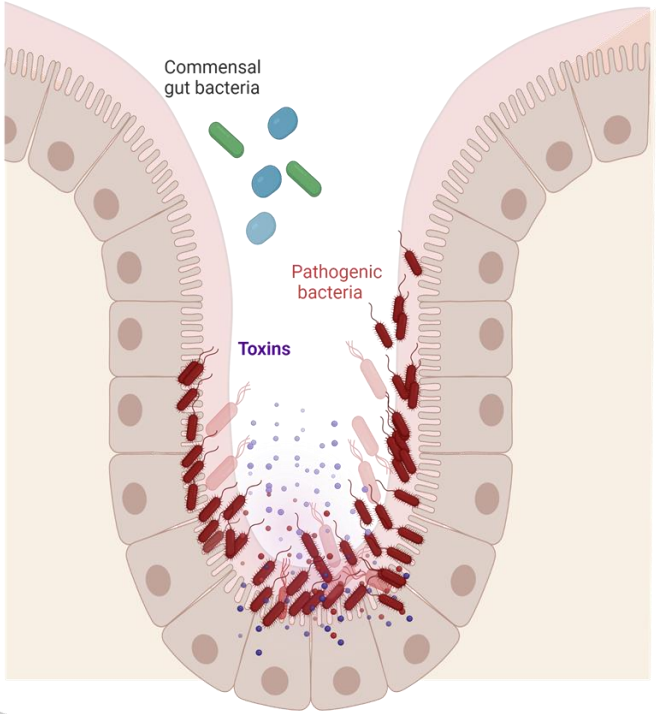
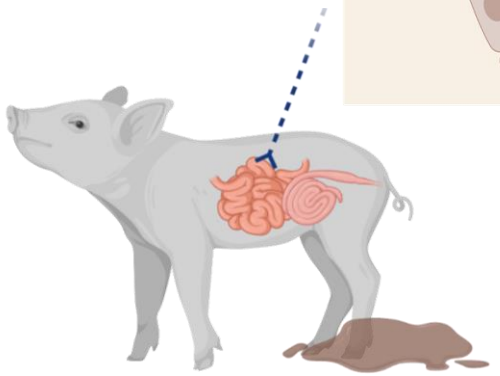
Predisposing factors

- Weaning stress
- Dietary change
- Undeveloped GIT
- Genetic





Enterotoxigenic *E. coli* (ETEC)

- Fimbriae: **F4, F18**
- Toxins: **LT, STa, STb**

~~Antibiotics~~
~~Zinc Oxide~~

Postweaning diarrhea (PWD)

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E. coli and postweaning diarrhea

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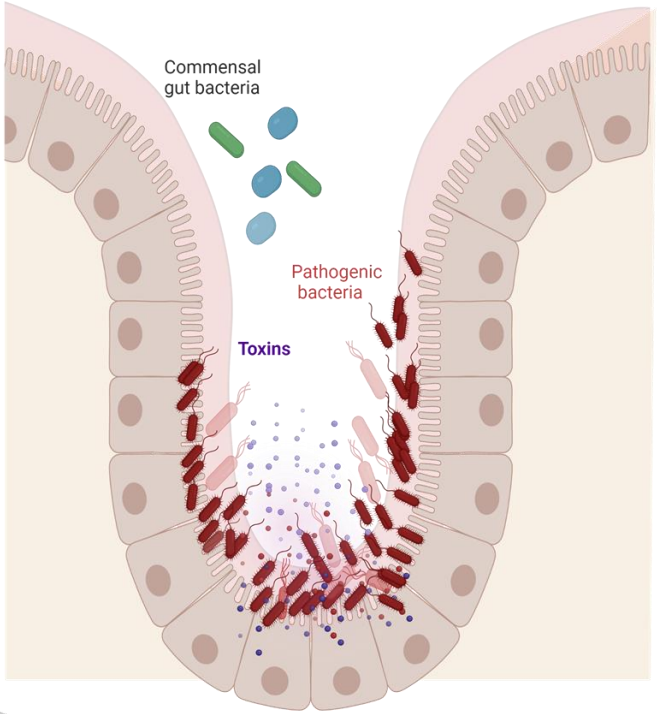

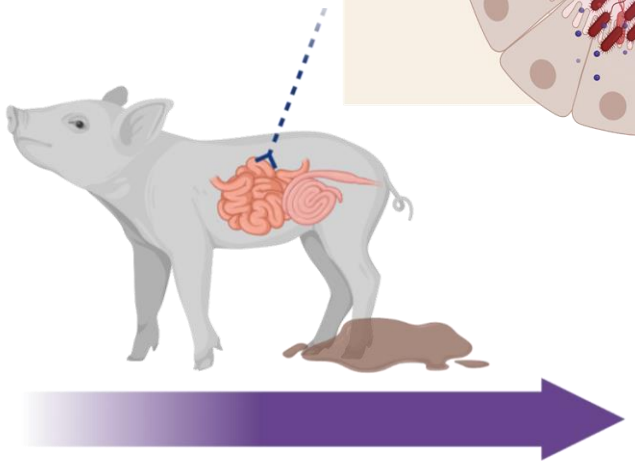

Postweaning diarrhea (PWD)

Enterotoxigenic *E. coli* (ETEC)

- Fimbriae: **F4, F18**
- Toxins: **LT, STa, STb**

~~Antibiotics~~
~~Zinc Oxide~~

?

Alternatives

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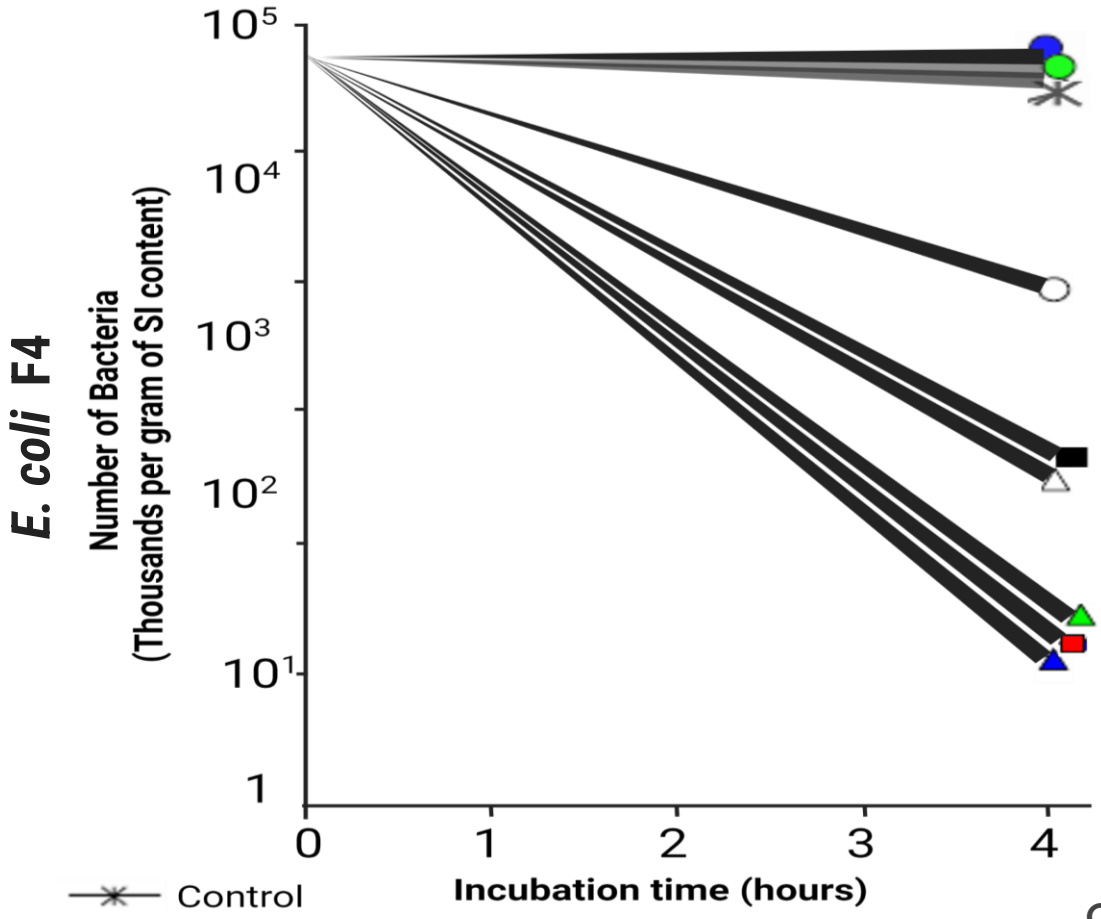
Some plants displayed synergistic antibacterial action (*in vitro*)



Wild Garlic (1%) + Lingonberries(1%)

Canibe, et al. 2018. 14th International Symposium on Digestive Physiology of Pigs

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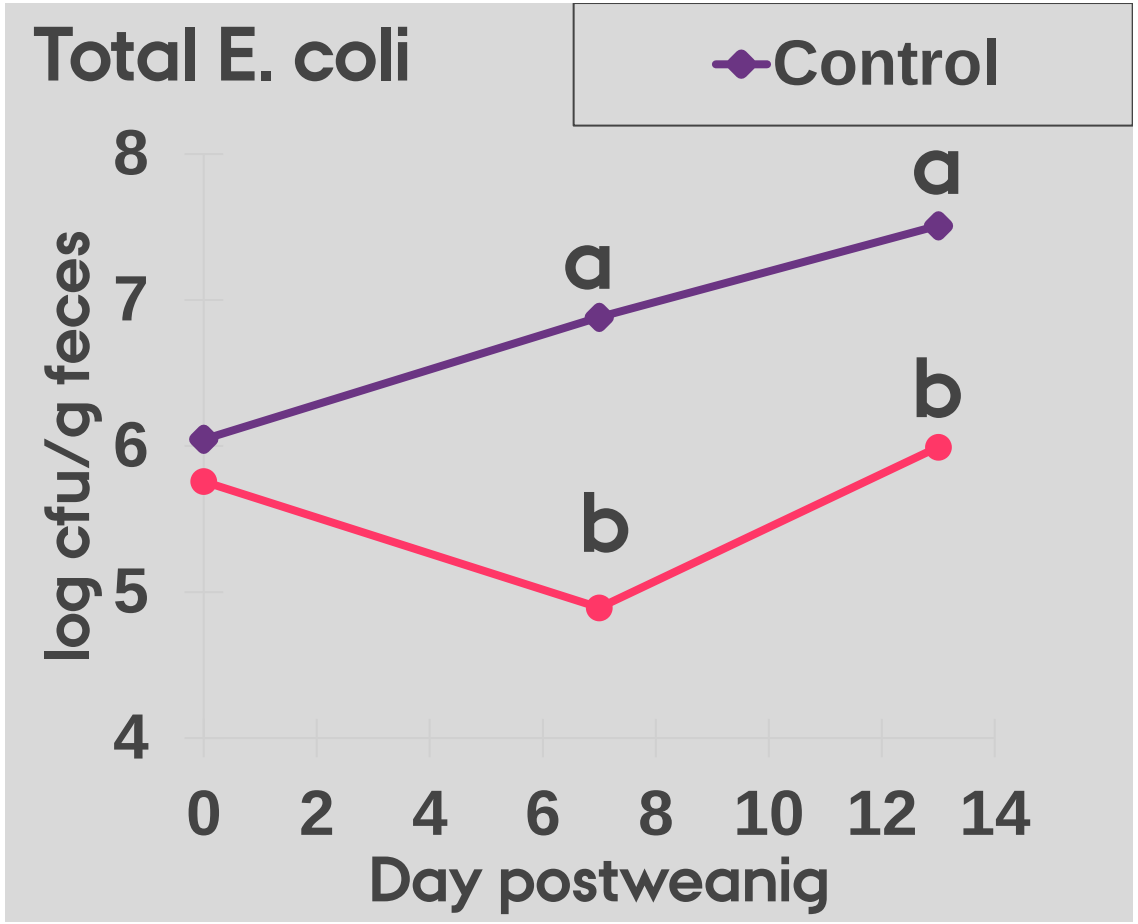
Plant reduced E coli (*in vivo* / non challenge)



Canibe, et al. 2018. 14th International Symposium on Digestive Physiology of Pigs

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Wild Garlic (1%) + Red Currant (1%)

✓ Antibacterial ✓ pH reduction

✓ Synergistic (less inclusion of both)

But...

- Scalability: both scarce

Canibe, et al. 2018. 14th International Symposium on Digestive Physiology of Pigs

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Replicate concept with widely available plants and efficacy testing

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pH reducing effect

Blackcurrant

Apple pomace

> Organic acids, polyphenols, dietary fiber

Antibacterial

Garlic

> Allicin, organosulfurs

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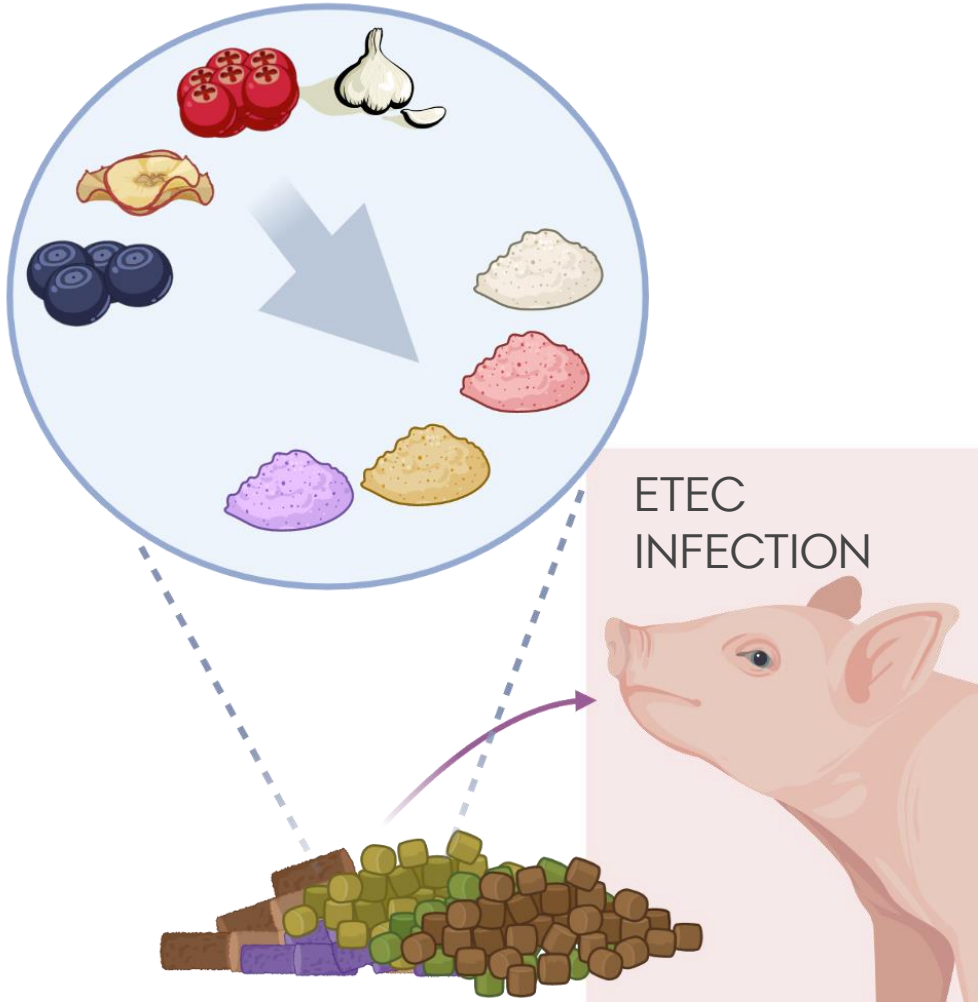
Hypothesis

The combination of **garlic and fruits** in the diet will inhibit ETEC growth on **ETEC-F18** challenged pigs without harming the gut microbiota, thereby preventing postweaning diarrhea.



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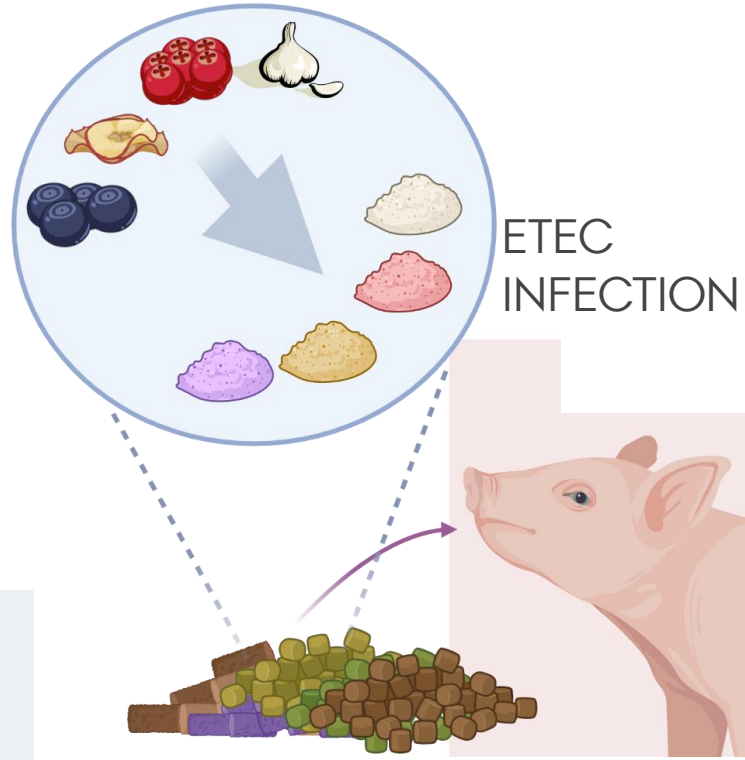
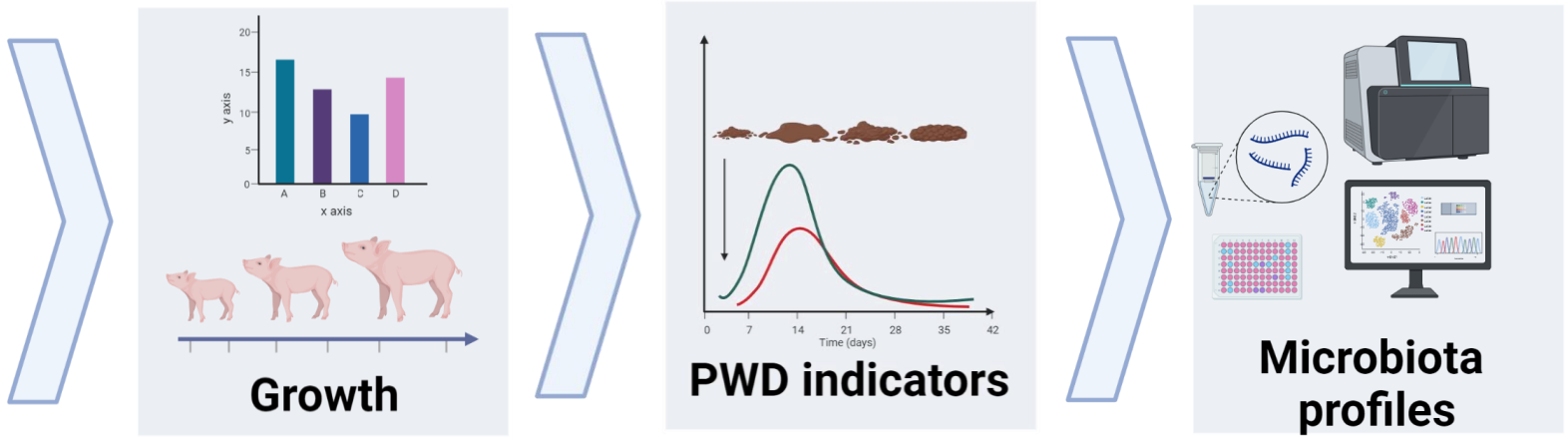
Objective

To evaluate the effects of including garlic+apple pomace or garlic+blackcurant in diets for ETEC F18-challenged weaned pigs (from organic farm) on growth performance, health status and fecal microbiota.





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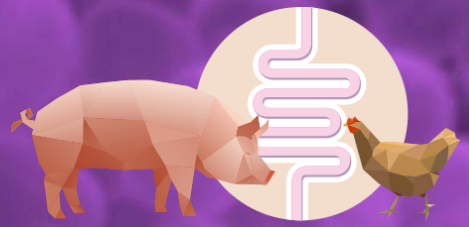

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02

Materials and Methods

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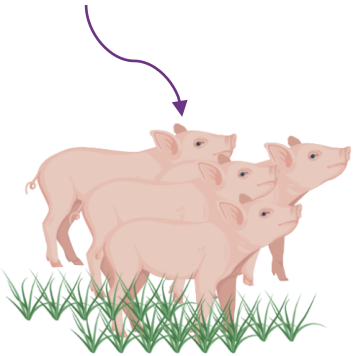
Experimental outline

+ ETEC receptors

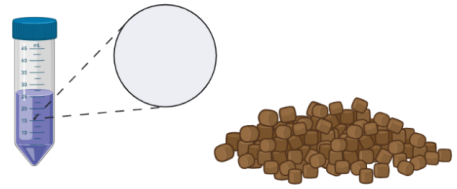
× 4



32 piglets

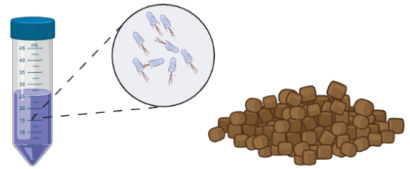
7 wk old

~20 kg



Saline solution

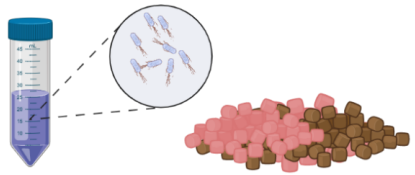
Standard Feed



10^9 CFU/ml ETEC

E. coli F18 infection

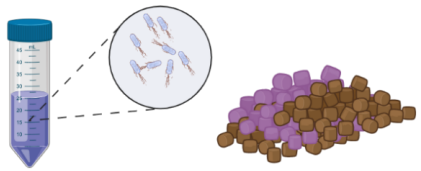
Standard Feed



10^9 CFU/ml ETEC

E. coli F18 infection

Garlic + Apple pomace (3%+3%)



10^9 CFU/ml ETEC

E. coli F18 infection

Garlic + Black Currant (3%+3%)

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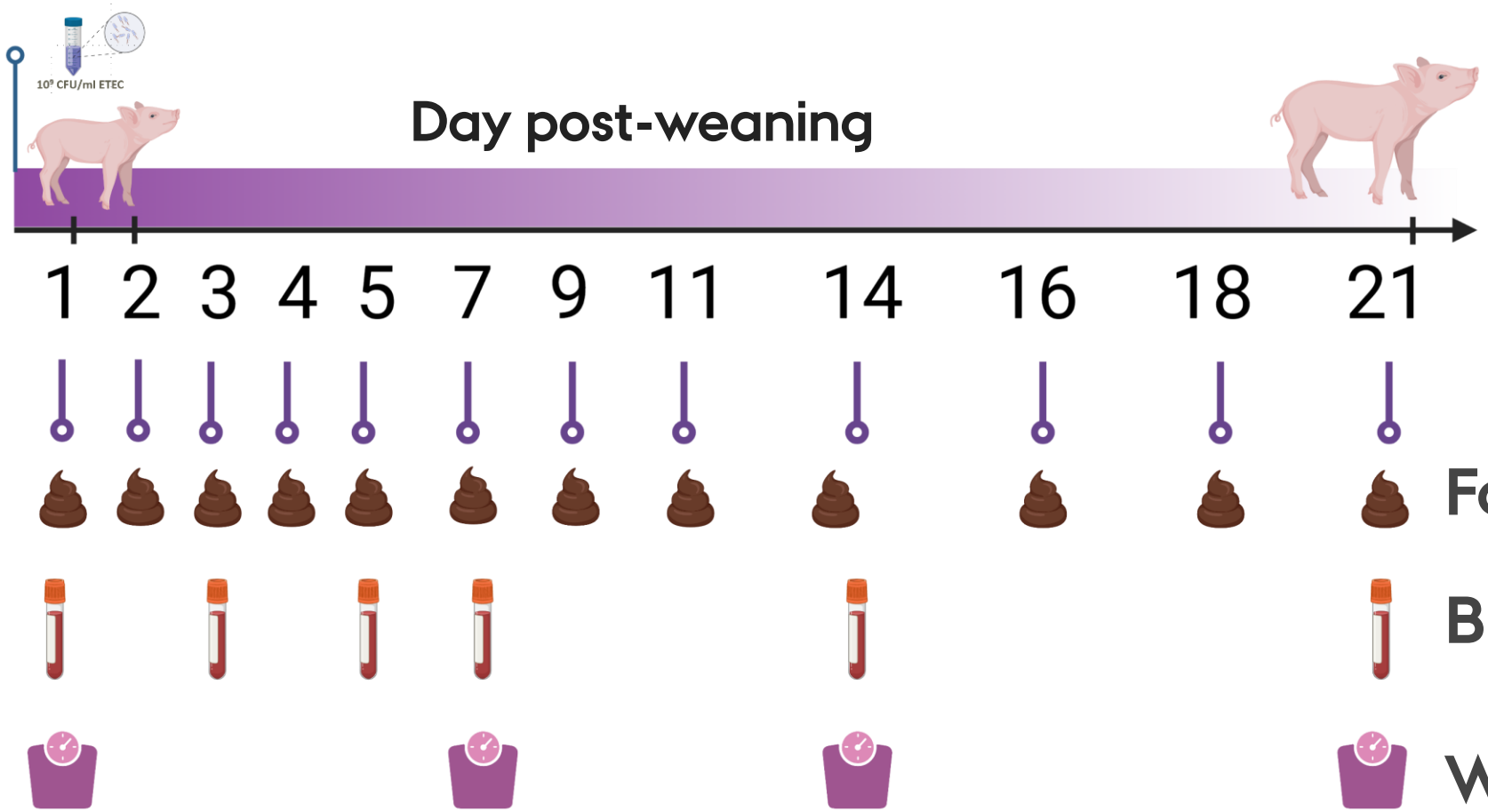
Experimental timeline



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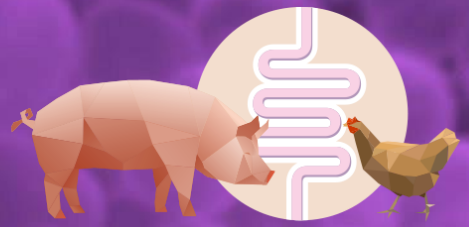

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03

Results

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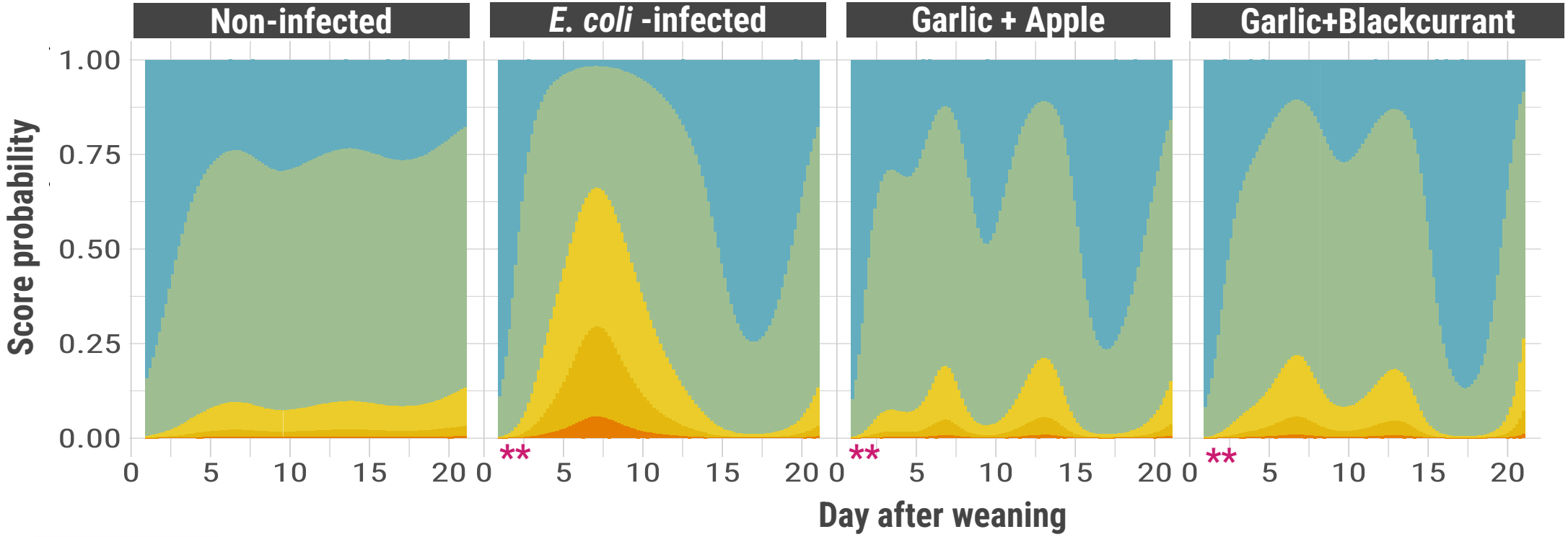
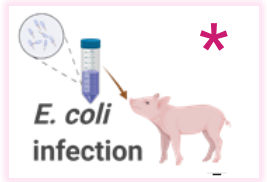
Plants supplementation reduced diarrhea occurrence

Fecal consistency score by day and treatment (1 to 7-point scale)

Treat × Day: *P* < 0.001





Fecal Score 1 2 3 4 5 6

Normal feces **Diarrheal feces**

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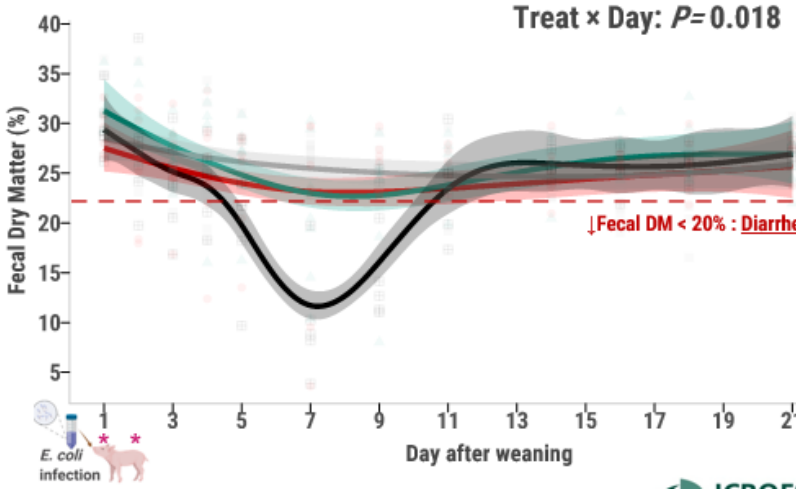
Non Infected

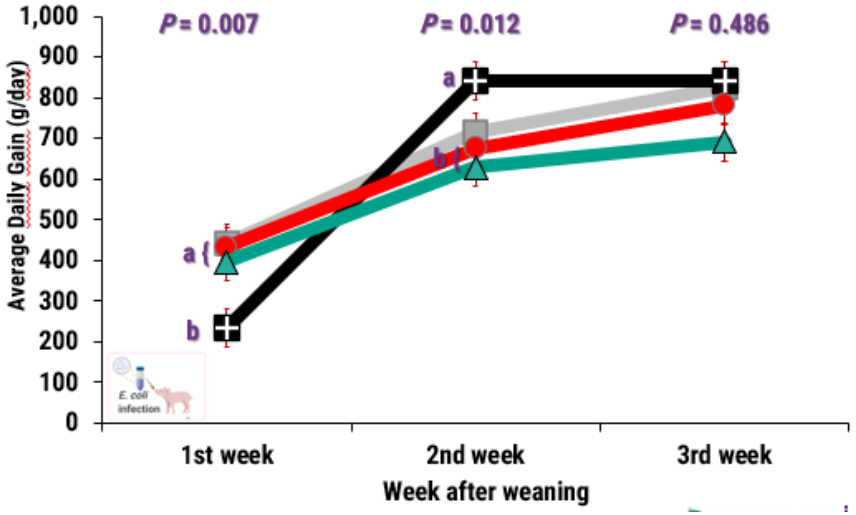
Garlic+Apple

E coli Infected

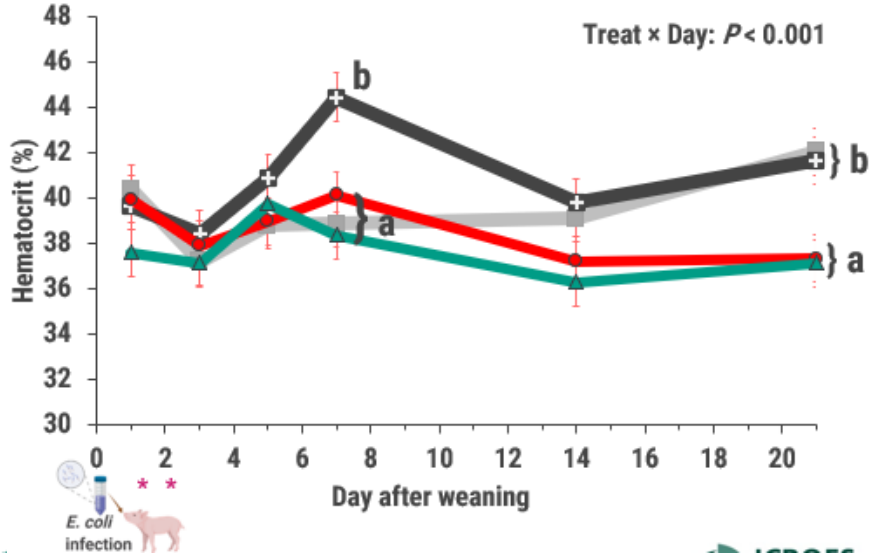
Garlic+Blackcurrant



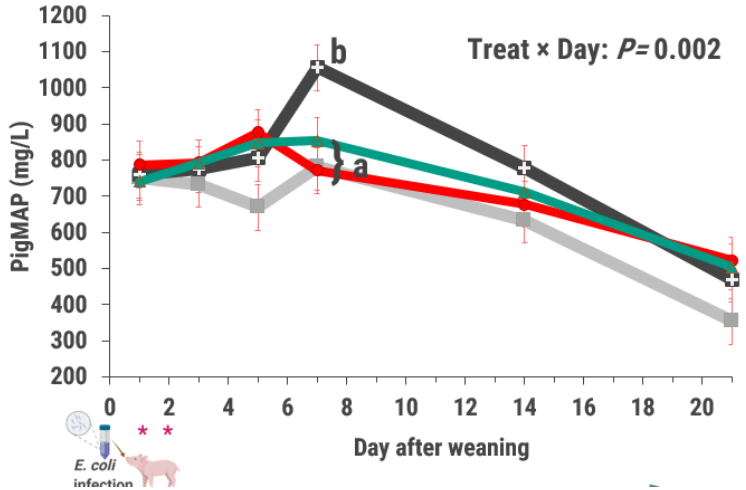
Faecal dry matter



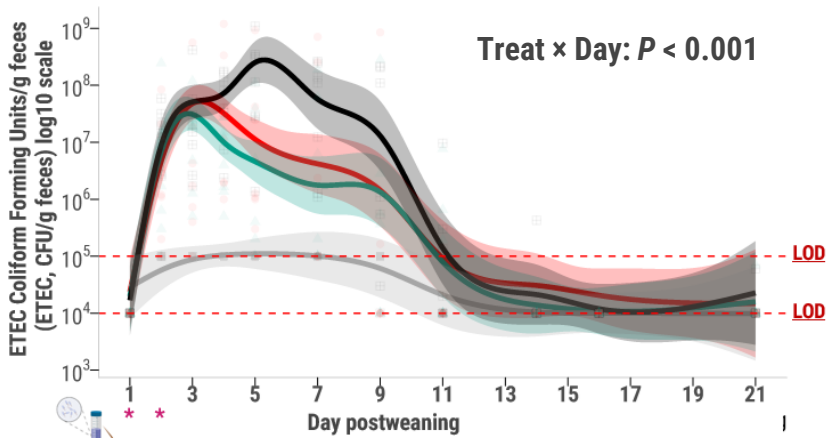
Average daily gain



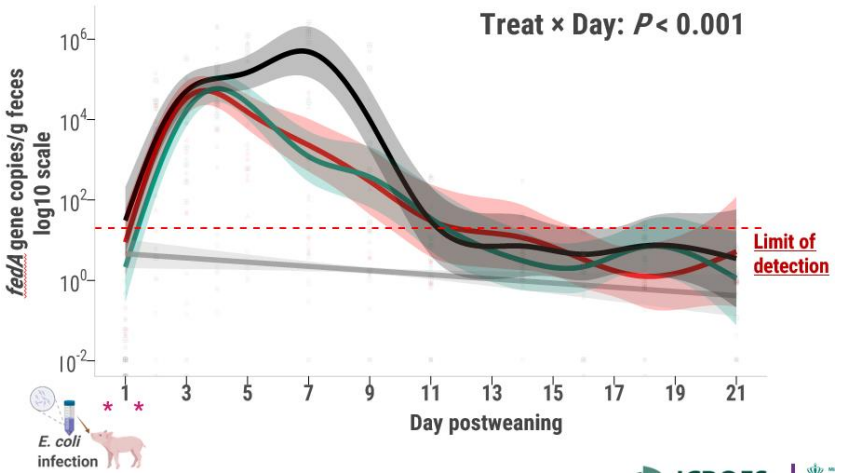
Haematocrit



PigMAP



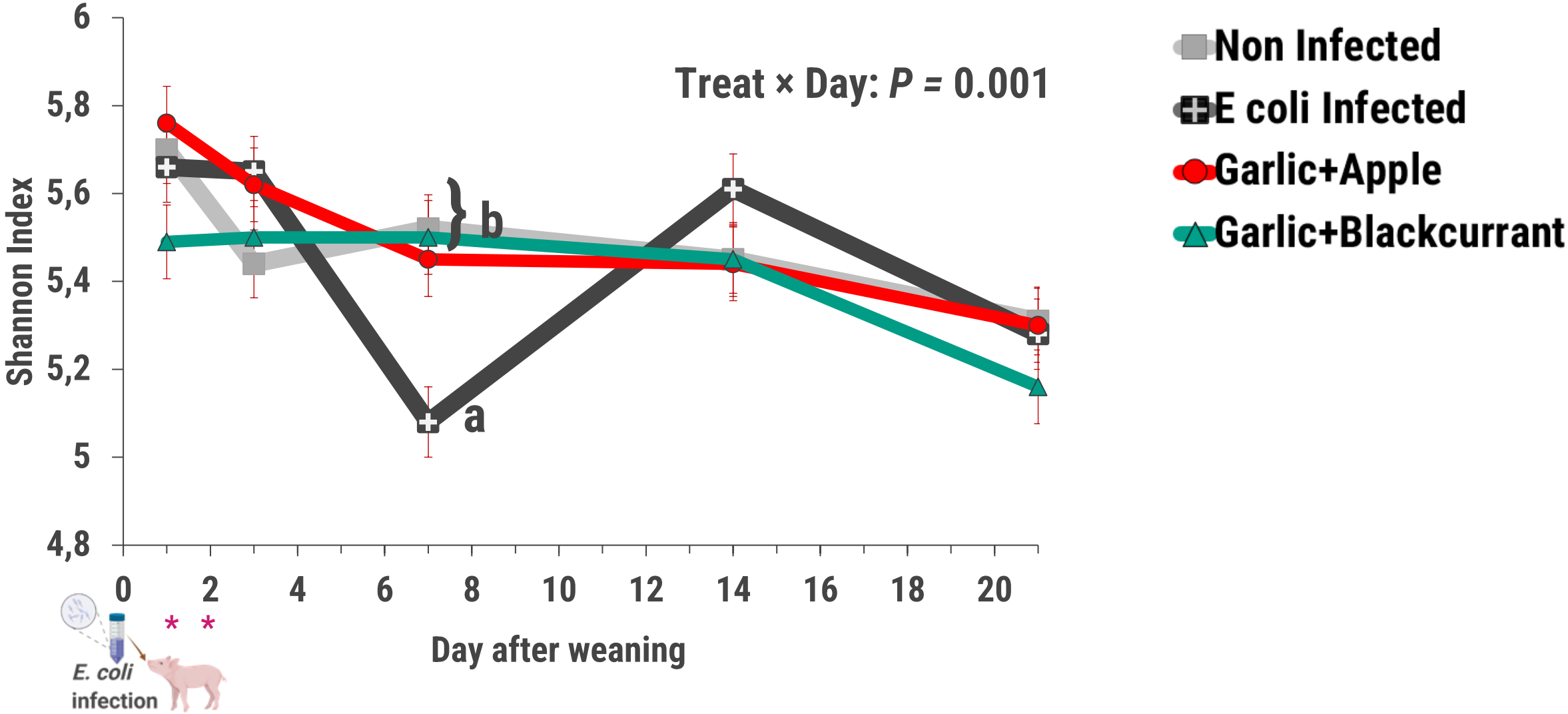
Haemolytic *E. coli*



F18 gene copies

Supplementation maintained the fecal microbiota richness and balance

Alpha diversity measure (Shannon Index)



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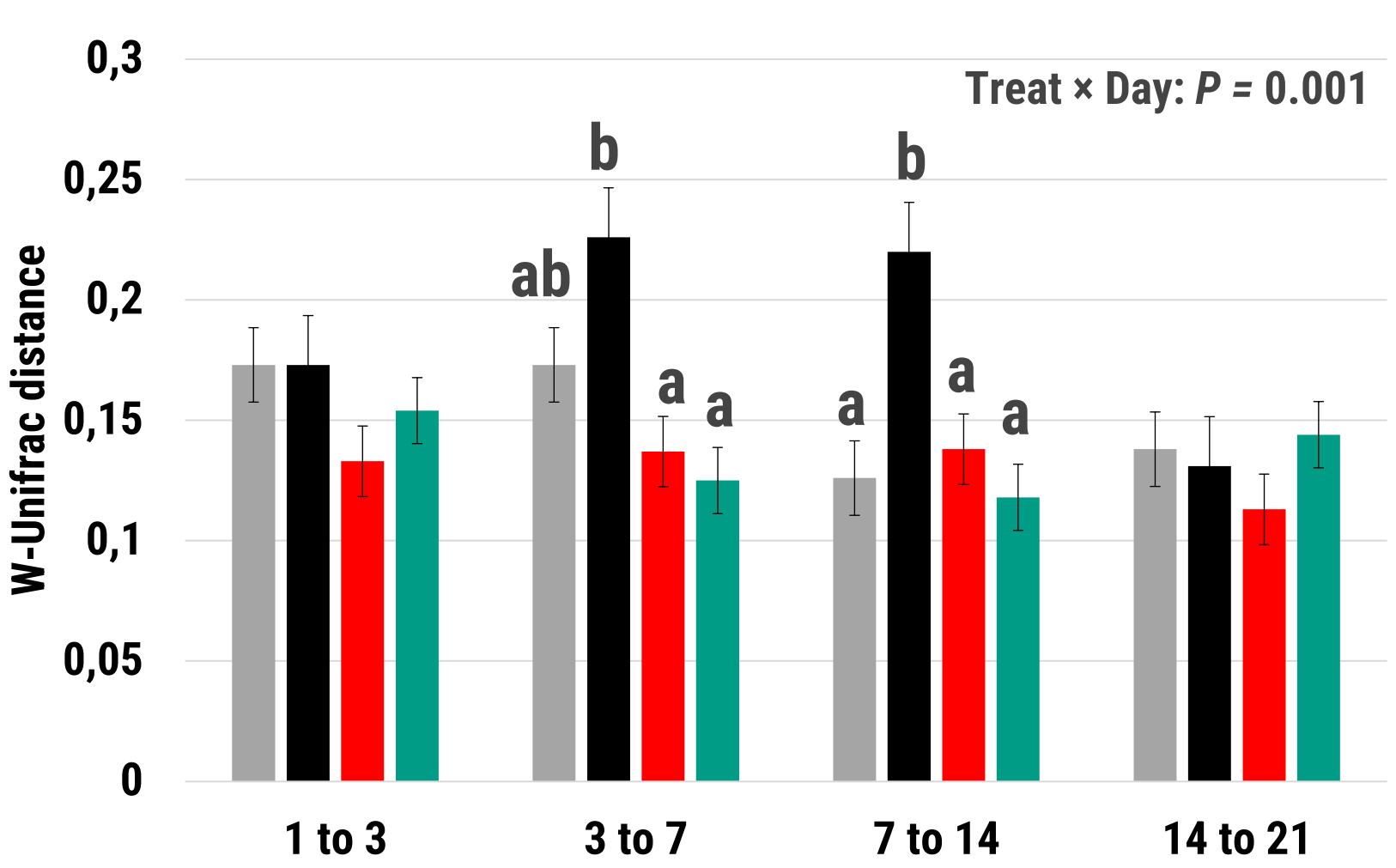
Supplementation promote a more stable microbiota

Beta diversity measure (1lag W-Unifrac distance)



Non Infected

E coli Infected

Garlic+Apple

Garlic+Blackcurrant

MICROBIOTA VOLATILITY:
How different is the microbial composition from previous observation
A lower number indicates a more stable microbiota

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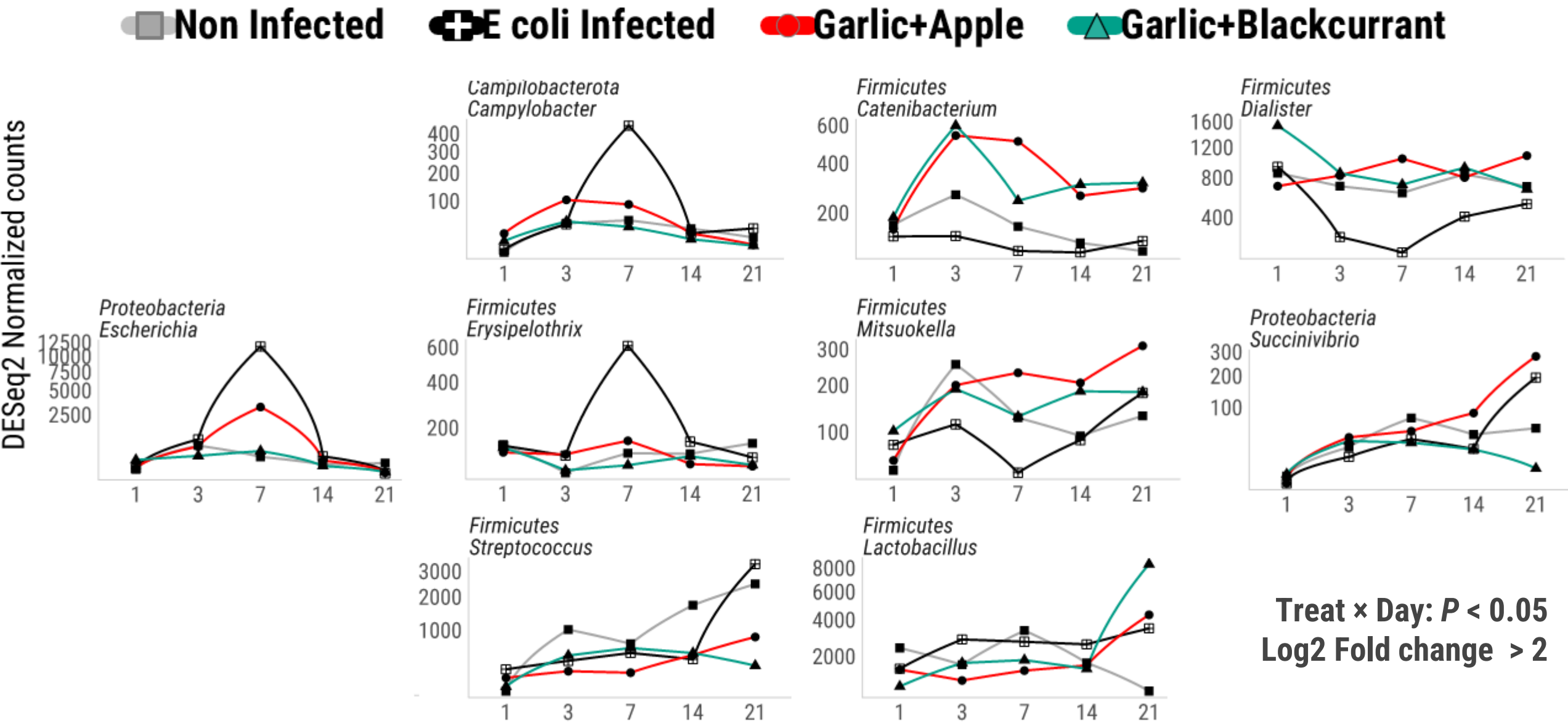
Differential abundance analyses in DESeq2 LRT time course experiment



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Conclusions

- **Garlic + Apple pomace** or **Garlic + Blackcurrant** (3% and 3%) in postweaning feed avoided *E. coli* growth
  - ✓ Had a reduced ETEC shedding in feces
  - ✓ Did not developed postweaning diarrhea
  - ✓ Had more diverse and stable microbiota

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THANK YOU!

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Do you have any questions?

Aarhus University

Kevin Jerez-Bogota

Jerezbogota@food.au.dk

monoguthealth.eu/

@MonoGutHealth

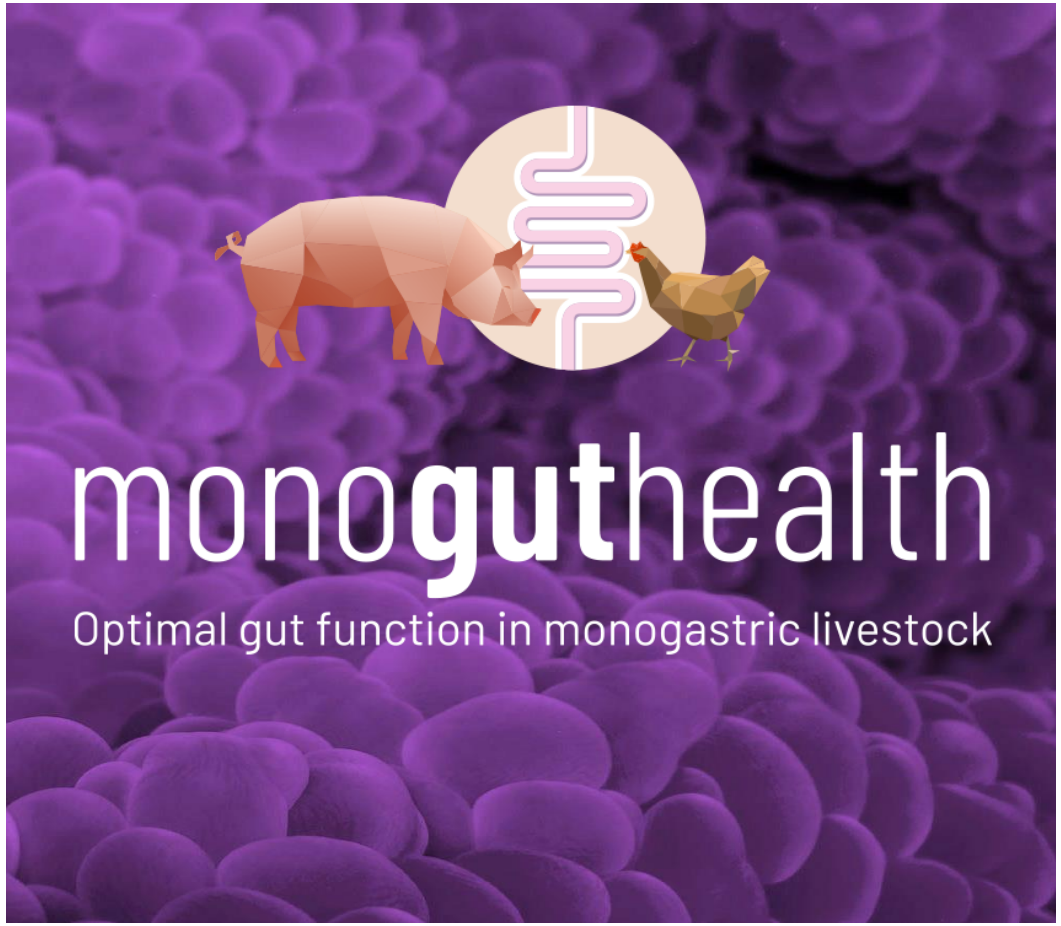
Front Vet Sci. 2023 Apr 3;10:1095160. doi: 10.3389/fvets.2023.1095160. eCollection 2023.

Antibacterial plant combinations prevent postweaning diarrhea in organically raised piglets challenged with enterotoxigenic *Escherichia coli* F18

Kevin Jerez-Bogota [1 2], Martin Jensen [1], Ole Højberg [2], Paul Cormican [3], Peadar G Lawlor [4], Gillian E Gardiner [5], Nuria Canibe [2]

Affiliations + expand

PMID: 37077951 PMCID: PMC10106643 DOI: 10.3389/fvets.2023.1095160



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Measurements and lab analyses

- **Growth and Intake**
- **Fecal samples**
  - ✓ Consistency score (1-7 scale; 4-7 diarrhea)
  - ✓ Dry matter (Freeze drying)
  - ✓ ETEC-F18 count (Blood agar + Agglutination test)
  - ✓ Genomic markers (qPCR: F18, STb)
  - ✓ 16s rRNA gene sequencing (Day 1, 3, 7,14, 21)
- **Blood samples (1, 3, 5, 7, 14, 21 d)**
  - ✓ Hematology, Acute Phase Proteins

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Calculations and Statistics

- **Generalized linear mixed models OR Generalized Additive models**
  - Fixed: Time ×Treatment
  - Random: Pig, Sow, Pen
  - Benjamini-Hochberg adjustment
- **16s gene sequencing reads**
  - DADA2
  - α-Diversity, β-Diversity
  - Dif. Abundance (Deseq2)

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