



Effect of a botanical-based feed additive *in vitro* on cultured enterocytes and *in vivo* on weaned piglets challenged with F4⁺ enterotoxigenic *Escherichia coli*

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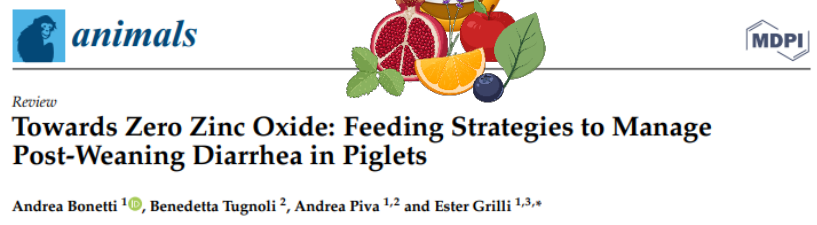
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Our blend of botanicals



1

DOI: 10.3390/ani11030642



Selection of candidate botanicals

2

DOI: 10.3390/antibiotics11081073



Identification of best candidates to control *E. coli* F4⁺ growth and virulence

3

DOI: 10.3389/fvets.2023.1141 frontiers | Frontiers in Veterinary Science

Botanicals as a zinc oxide alternative to protect intestinal cells from an *Escherichia coli* F4 infection *in vitro* by modulation of enterocyte inflammatory response and bacterial virulence

Andrea Bonetti¹, Andrea Piva^{1,2}, Ester Grilli^{1,3,*}

Identification of best candidates to improve intestinal cells integrity and resilience

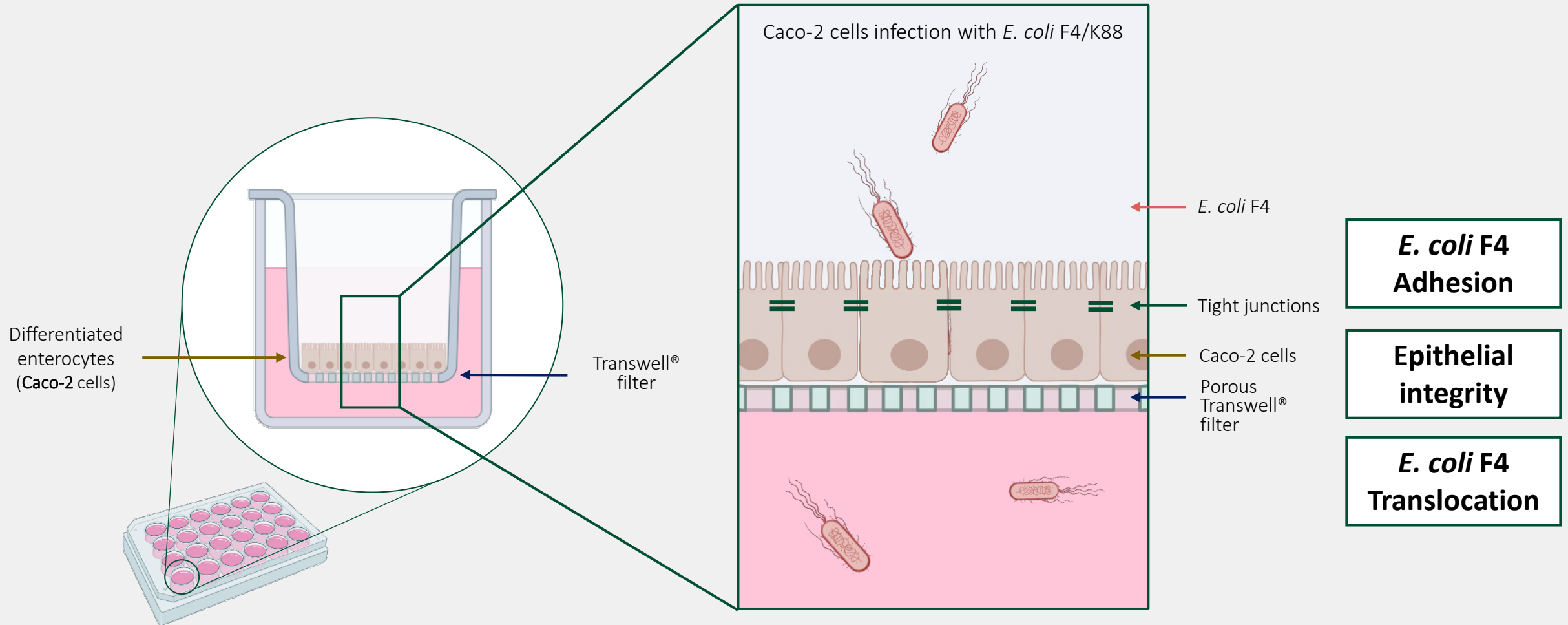


Blend of selected botanicals (BOT)

Testing its efficacy:

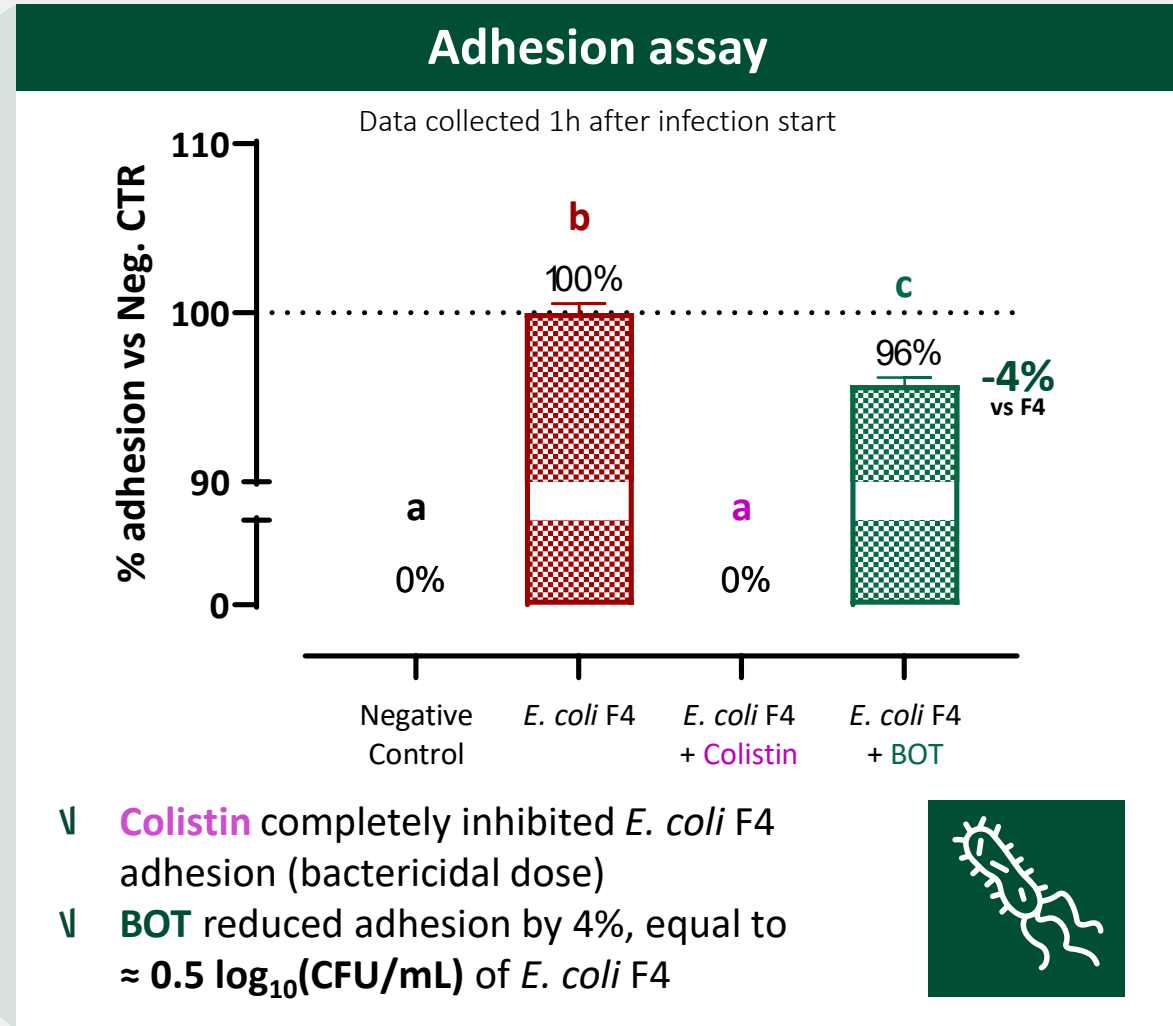
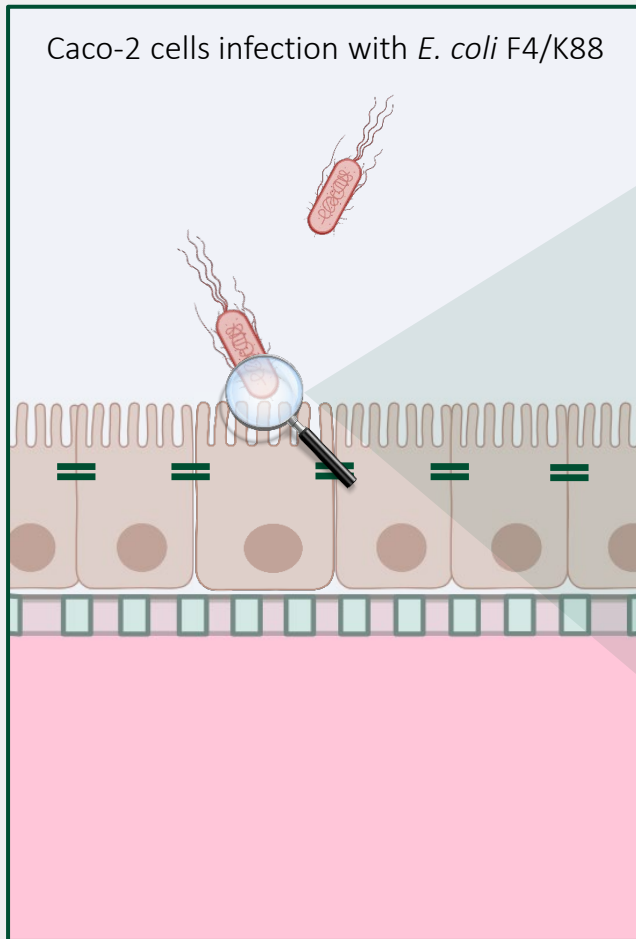
- ✓ *in vitro* on cultured enterocytes
 - ✓ *in vivo* on weaned piglets
- challenged with F4⁺ enterotoxigenic *Escherichia coli*

In vitro validation – an F4 challenge model



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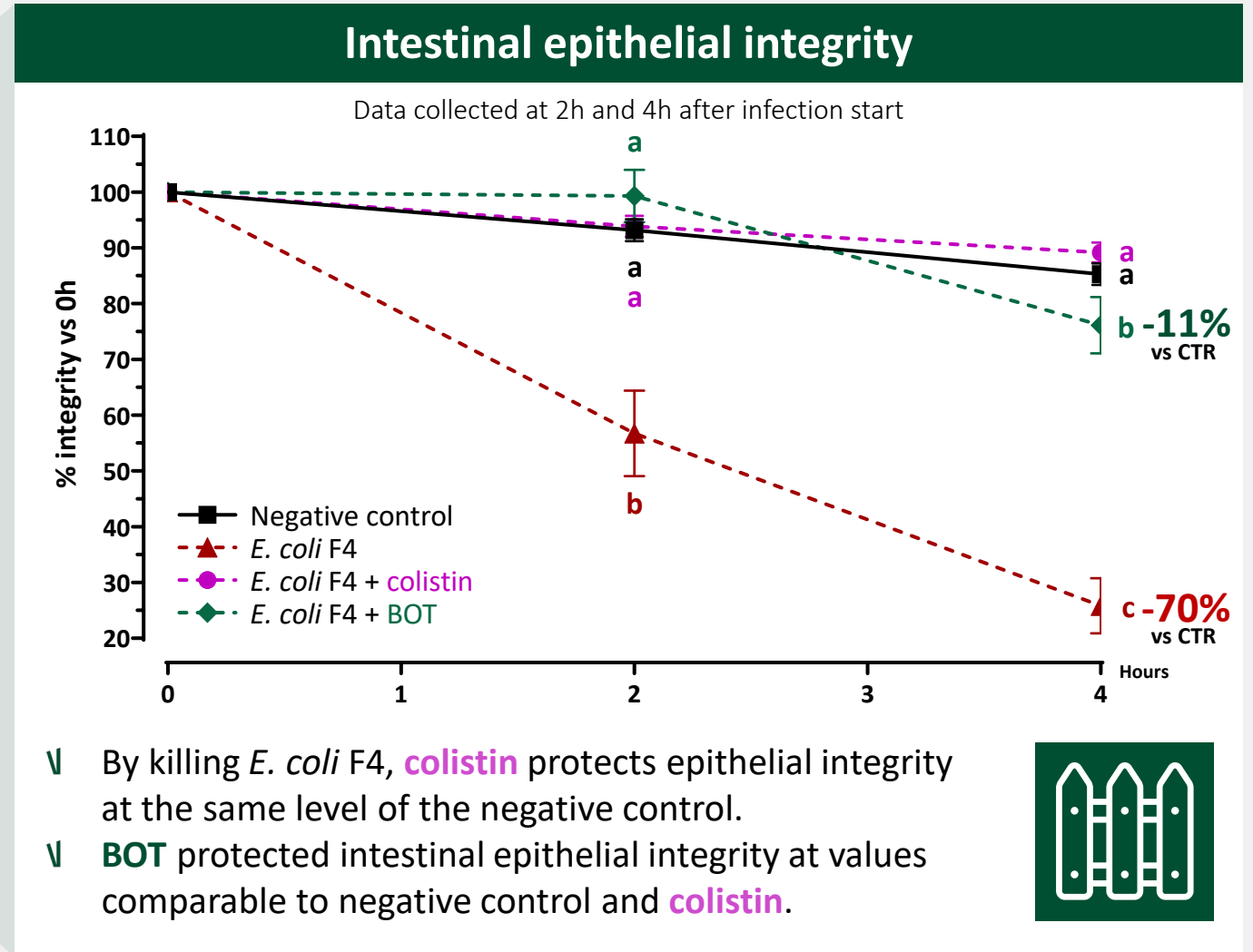
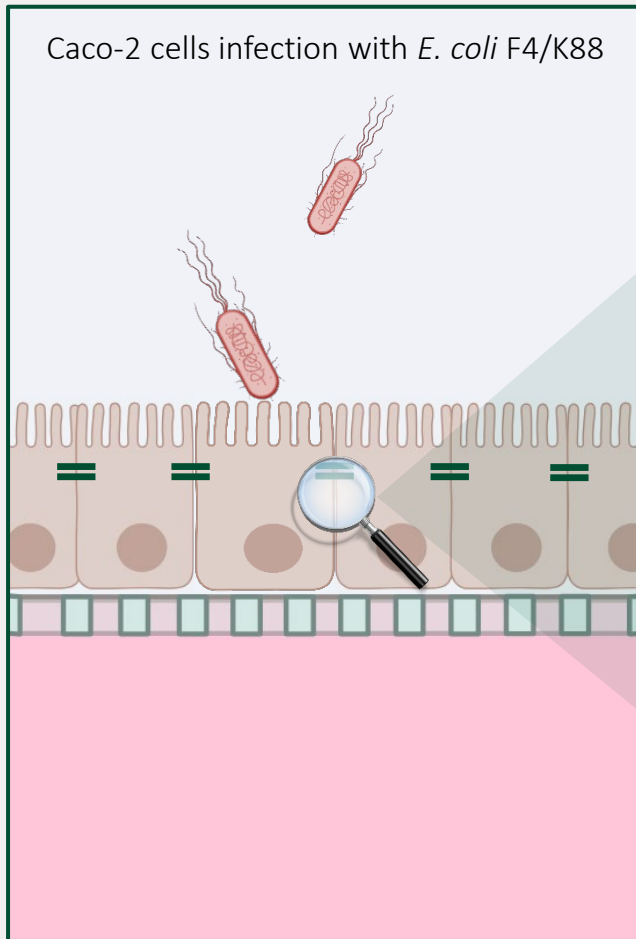
In vitro: F4 adhesion



Mean $\pm$ SEM, n=7.
Data analyzed with ANOVA ($P < 0.05$)

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In vitro: enterocyte integrity



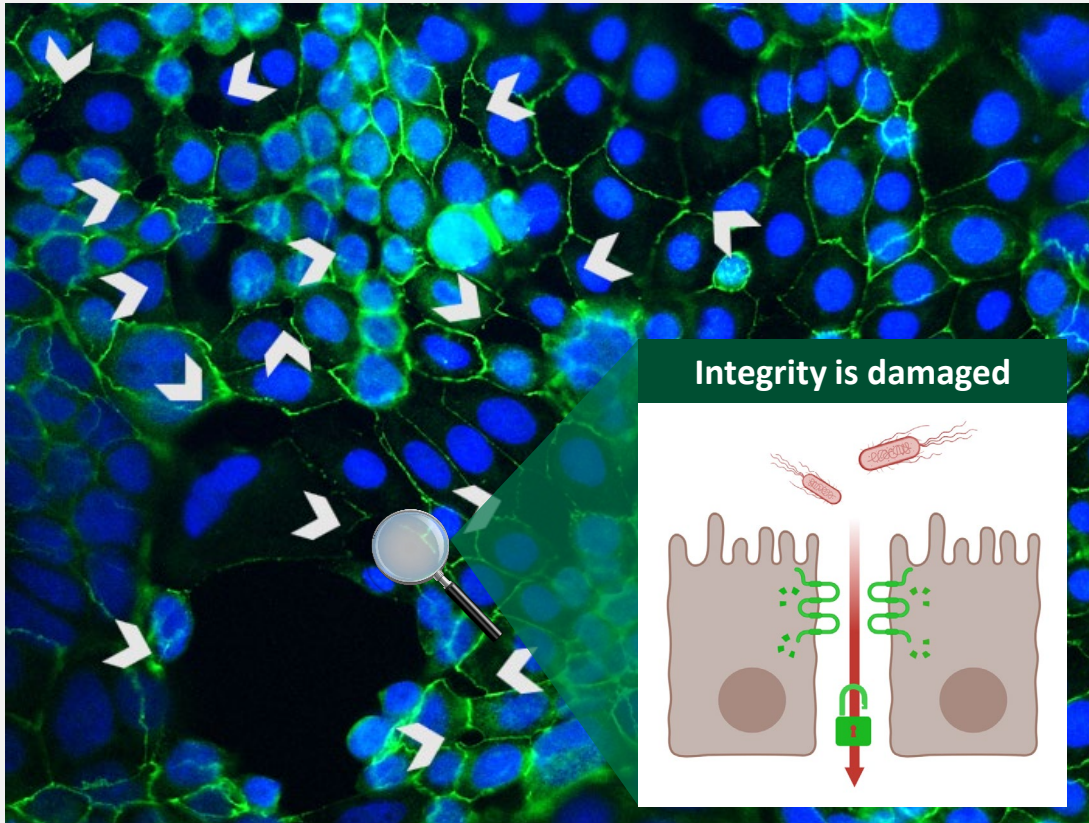
Mean±SEM, n=7.
Data analyzed with ANOVA ($P<0.05$)

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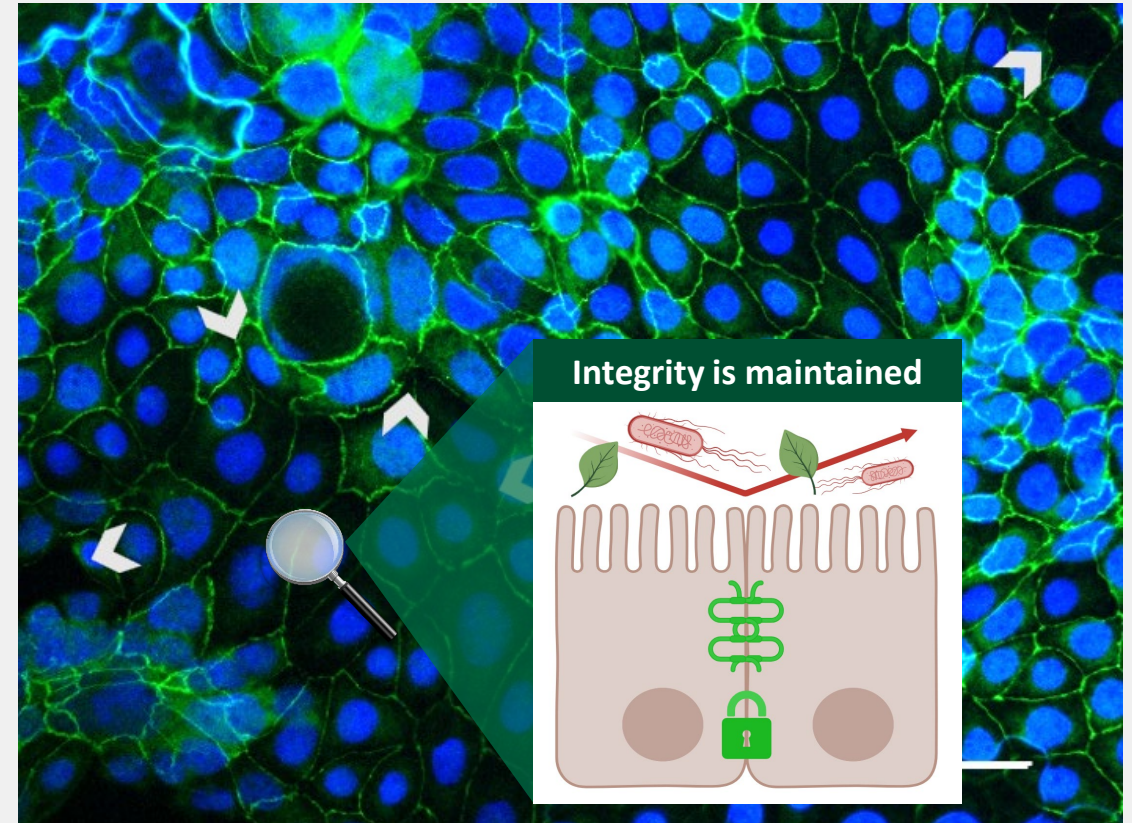
In vitro: a visual confirmation



E. coli F4⁺



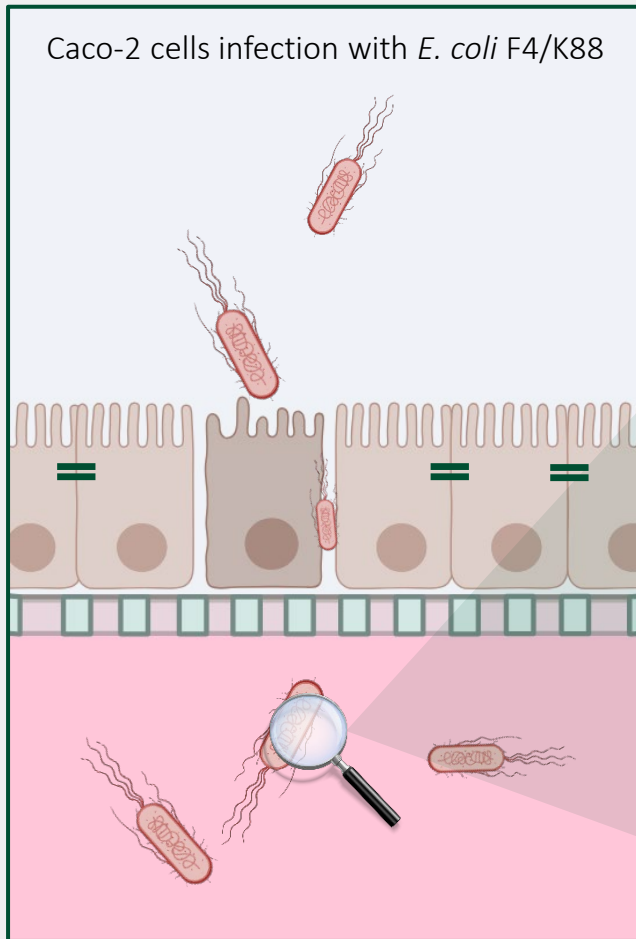
E. coli F4⁺ + BOT



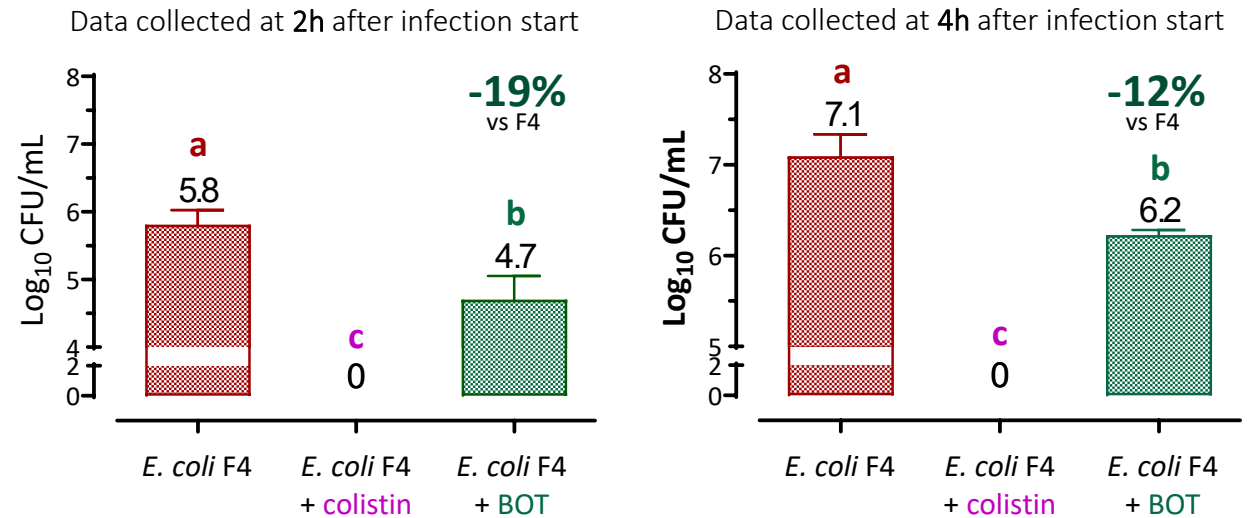
Immunofluorescence staining on intestinal Caco-2 cells challenged with *E. coli* F4 and treated with BOT.
DAPI (Blue): cell nuclei; FITC (Green): ZO-1 (tight junction) | Arrow identify areas of abnormal ZO-1 disposition, or loss of cells.

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In vitro: F4 translocation



Bacterial translocation across intestinal epithelium



▼ BOT reduction of bacterial translocation:

▼ At 2h: $-1.1 \log_{10}(\text{CFU/mL})$ (-19%) → 10 times less *E. coli* F4

▼ At 4h: $-0.9 \log_{10}(\text{CFU/mL})$ (12%)

▼ Colistin completely inhibited bacterial translocation (bactericidal dose)



Mean $\pm$ SEM, n=7.

Data analyzed with ANOVA ($P < 0.05$)

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In vivo validation – an F4 challenge model



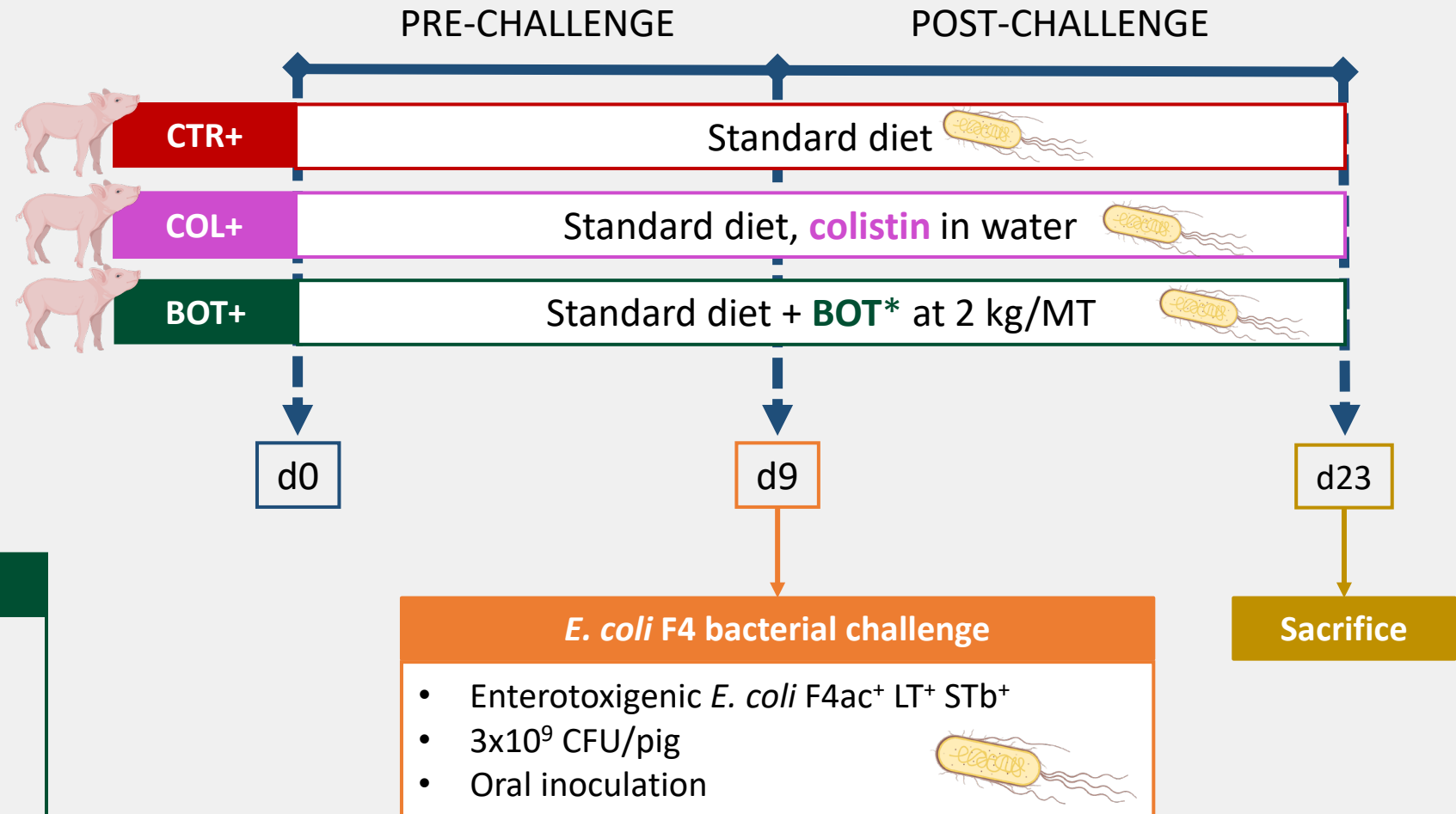
Trial details

- ✓ Weaning pigs (±31 days-old)
- ✓ 30 piglets
- ✓ 6 box (5 pigs/box)
- ✓ 3 experimental groups
- ✓ 10 pigs/group
- ✓ Standard diet with no AGPs, no pharmacological ZnO, no other feed additives



Data collected

- ✓ Growth performance
- ✓ Fecal consistency
- ✓ *E. coli* F4 fecal counts
- ✓ *E. coli* F4 intestinal virulence (end of trial)
- ✓ Intestinal health status (end of trial)



*BOT was added in the complete feed in a microencapsulated form

In vivo validation – growth performance



	CTR+	COL+	BOT+	SE	P value	
BW – d0 (kg)	9.0	9.1	9.1	0.3	0.94	
Before challenge (d0-9)						
BW – d9 (kg)	11.0	10.7	11.5	0.3	0.19	
ADG (g/pig/day)	251	206	296	31	0.15	
ADFI (g/pig/day)	271	260	331	26	0.31	
FCR	1.10^a	1.26^b	1.12^a	0.02	0.03	
After challenge (d9-23)						
BW – d14 (kg)	13.7	13.7	14.8	0.4	0.06	+ 1.1 kg
ADG (g/pig/day)	479	529	576	30	0.11	
ADFI (g/pig/day)	688^a	790^b	816^b	14	0.04	
FCR	1.45	1.50	1.41	0.04	0.51	
Overall (d0-23)						
BW – d23 (kg)	18.6	18.6	20.1	0.5	0.09	+ 1.5 kg
ADG (g/pig/day)	380	414	478	26	0.06	
ADFI (g/pig/day)	528^a	599^{ab}	641^b	9	0.02	
FCR	1.39	1.45	1.34	0.06	0.51	

Mortality:
2 pigs in CTR+; 1 pig in COL+, 1 pig in BOT+

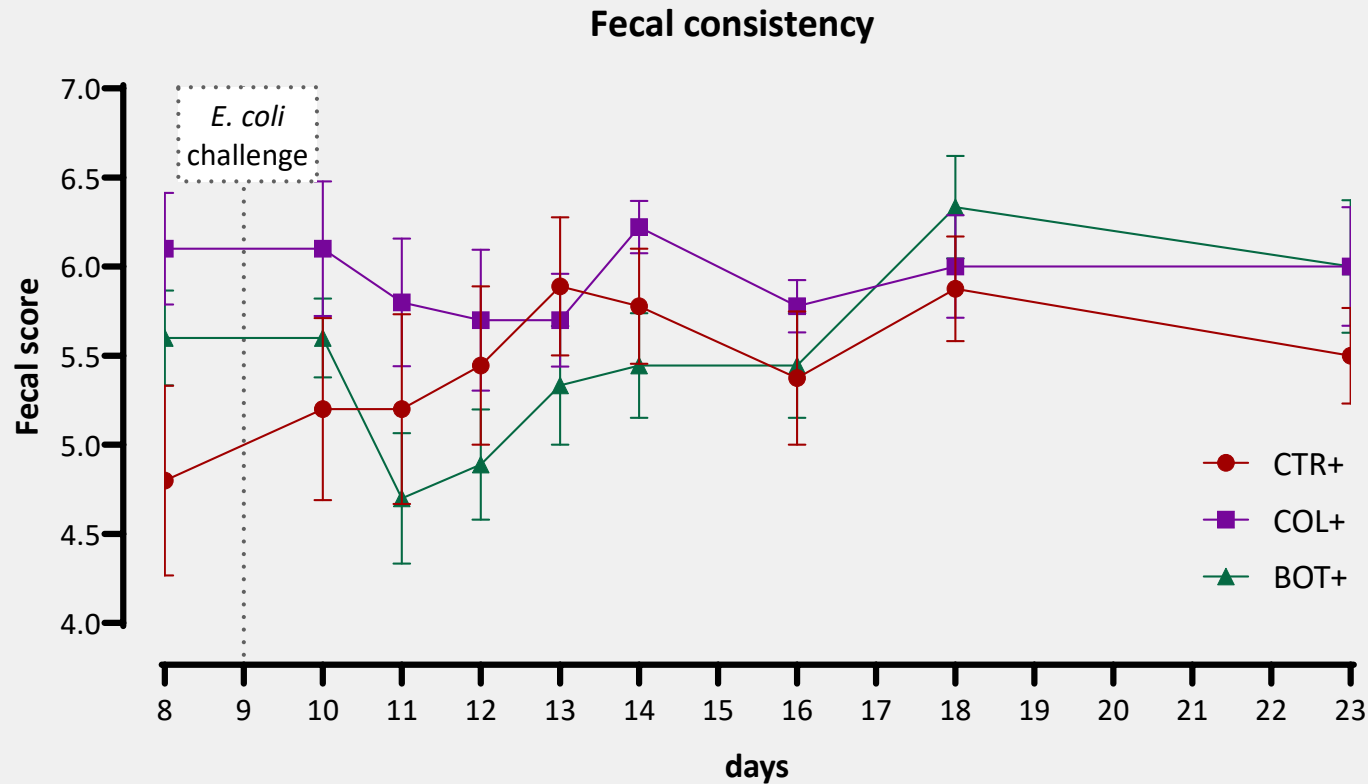
d9:
E. coli F4⁺ challenge

∨ **BOT** tended to improve growth performance compared to both CTR+ and COL+ at d14 and d23

Data analyzed with ANCOVA (BW covariate, $P < 0.05$)

^{ab} Values with different superscript within a row indicate significant differences ($P \leq 0.05$)

In vivo validation – fecal score



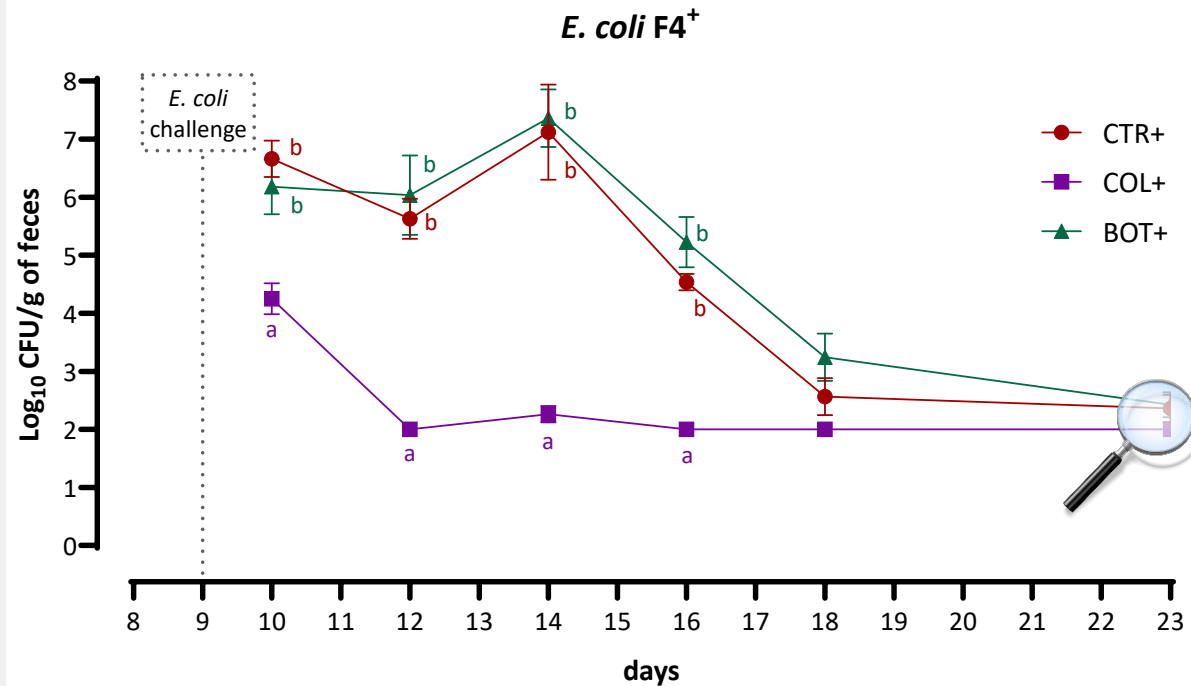
Score	Description
9	Lumpy
8	Firm and shaped with structure
7	Firm and shaped
6	Solid dropping without structure
5	Smooth
4	Like custard
3	Water-thin faeces
2	Severe water-thin diarrhea

∨ No significant effects on fecal consistency despite the challenge

In vivo validation – *E. coli* counts and virulence



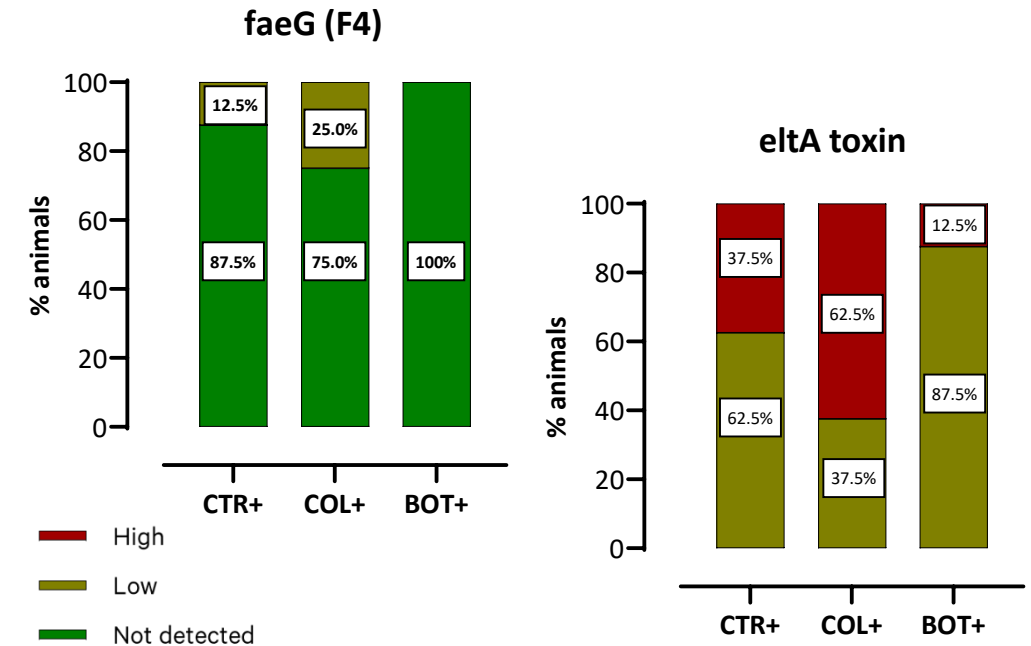
E. coli F4⁺ counts in feces



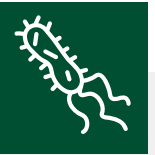
- Only colistin reduced *E. coli* F4⁺ fecal shedding
- CTR and **BOT** had similar *E. coli* F4⁺ fecal shedding

Pathogen side

E. coli F4⁺ virulence in ileum digesta



- BOT** reduced the expression of *E. coli* F4⁺ adhesin and diarrhea toxin in ileum digesta



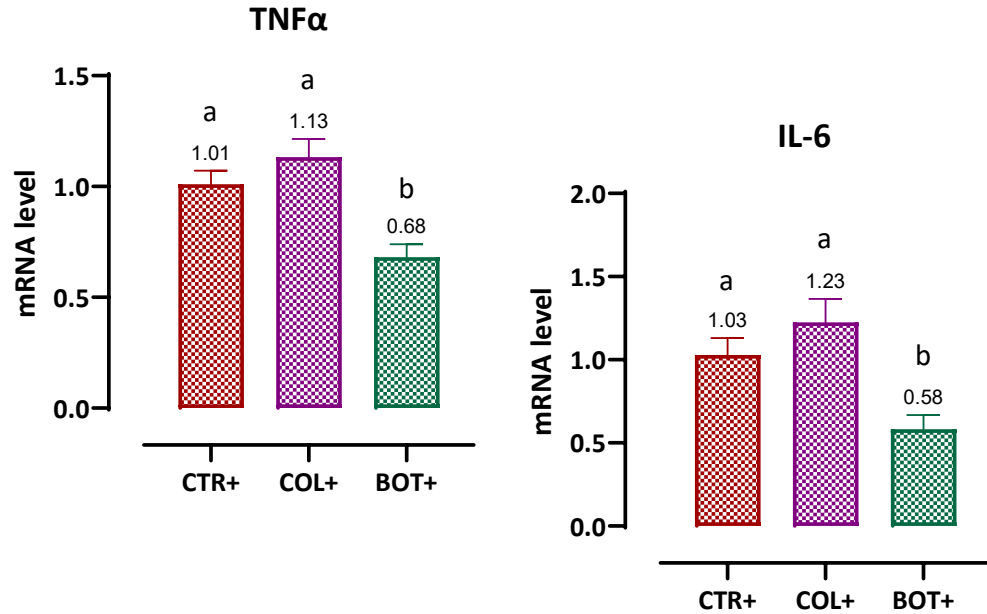
^{ab} Values with different superscript within a time-point indicate significant differences ($P \leq 0.05$)

In vivo validation – *E. coli* counts and virulence



Host side

Inflammatory markers in ileum mucosa

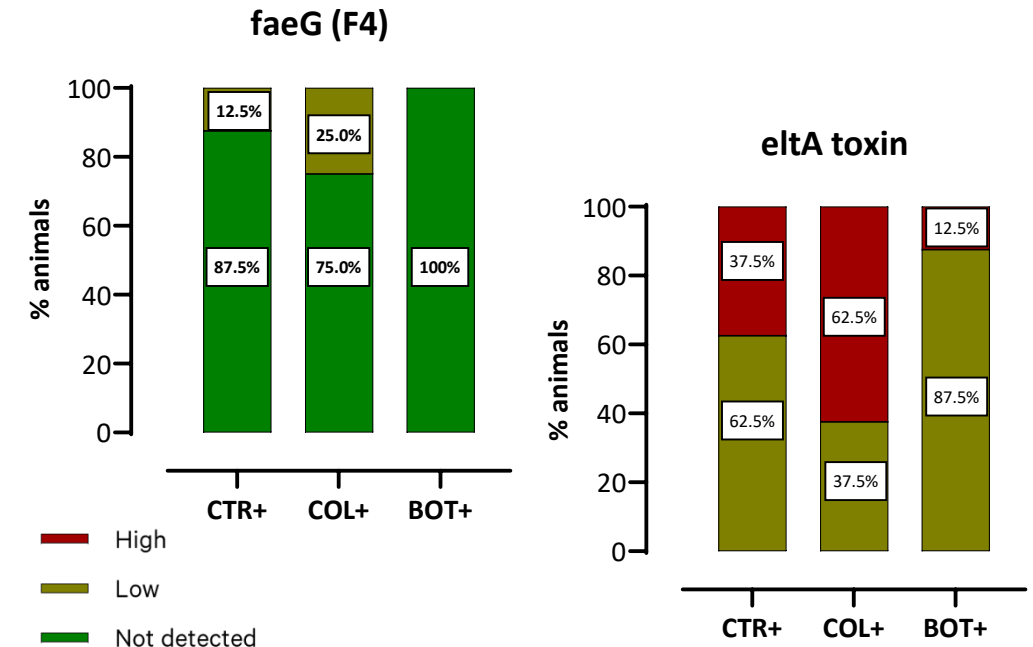


▼ **BOT** reduced the expression of pro-inflammatory cytokines in ileum mucosa

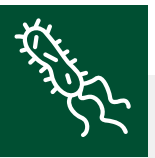


Pathogen side

E. coli F4⁺ virulence in ileum digesta



▼ **BOT** reduced the expression of *E. coli* F4⁺ adhesin and diarrhea toxin in ileum digesta



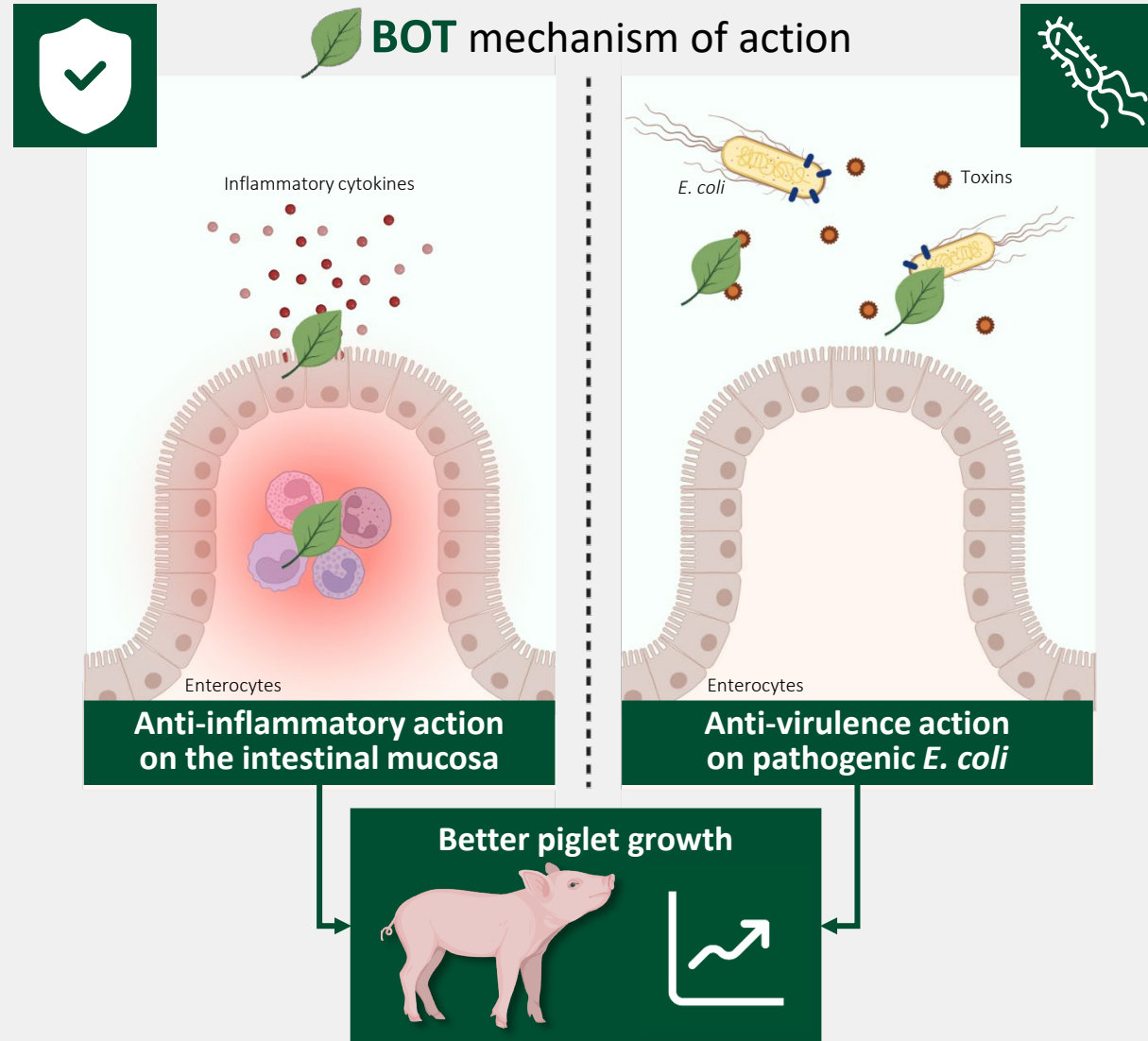
^{ab} Values with different superscript within a time-point indicate significant differences ($P \leq 0.05$)

Conclusion



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Vetagro's Talk – Tomorrow at lunchtime



***“From mitigating environmental impact to antimicrobial reduction:
how botanicals can improve animal production sustainability”***



Benedetta Tugnoli

*PhD - Global Technical
Innovation Manager*



Sarda Room - 12:45 – 13:45
Palazzo Affari, Floor -1



Richard Paratte

*PhD - Global Ruminant
Technical Manager*



**A lunchbox
will be offered!**

Thank you for your attention

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For more information about

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