Impact of live yeast on the intestinal transcriptome profile of weaned pigs challenged with ETEC

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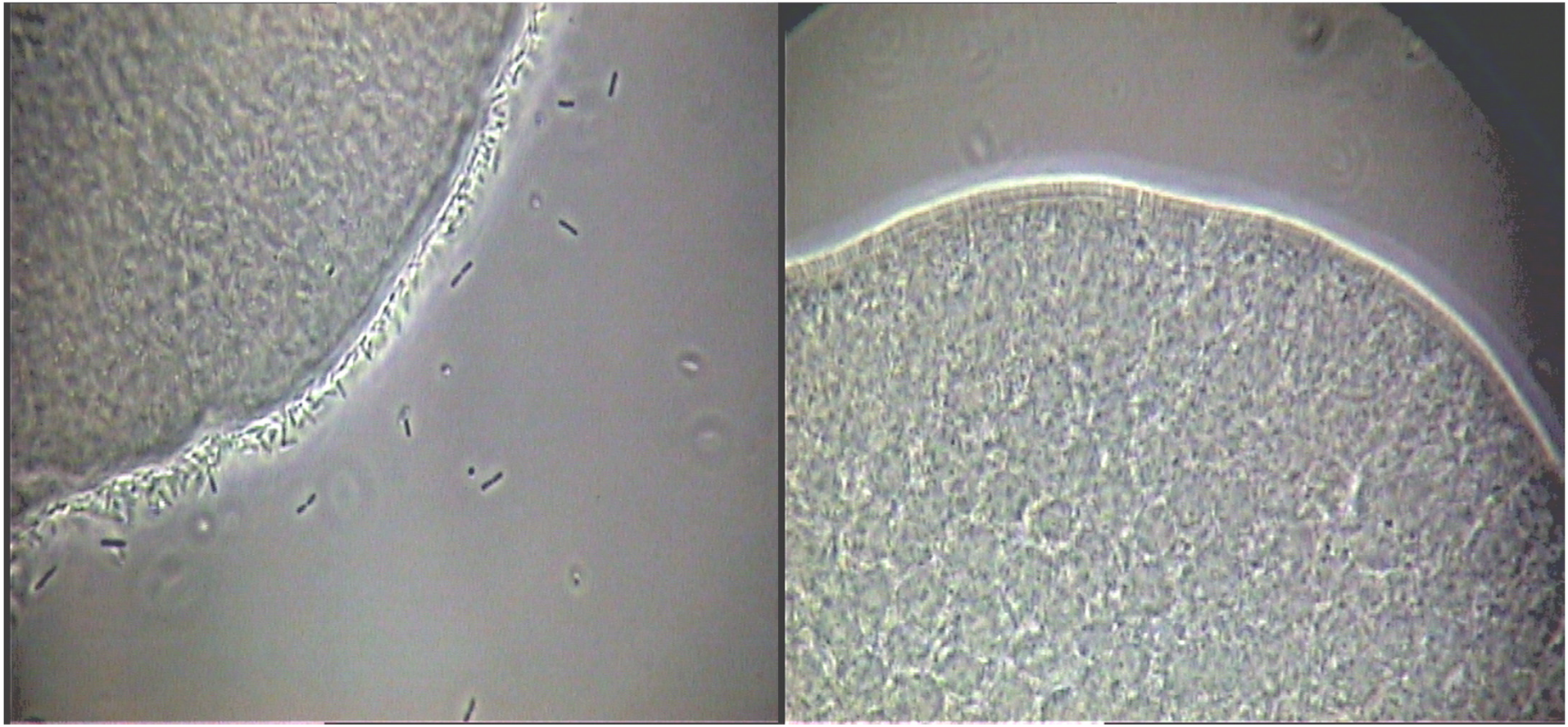
BACKGROUND

- The enterotoxigenic *E.coli* (ETEC) expressing the K88(F4) fimbrial antigens is one of the most important agents causing the neonatal diarrhoea and post-weaning diarrhoea in pigs

- The susceptibility to this colibacillosis depends on the presence of receptors for the fimbriae of ETEC in pig intestine.

- Susceptibility can be assessed by an *in vitro* test of *E. coli* F4 adhesion to intestinal tissue or receptors

Specific test to assess the presence of the specific receptors for ETEC F4ac on the surface of intestinal epithelium (phenotype)



Susceptible subject (F4 R^+ class 3) **Not susceptible subject (F4 R^- class 0)**

BACKGROUND

Oral challenge with live Enterotoxigenic *E.coli* F4

The oral challenge with ETEC has been often used in feeding trials on piglets to evidence the ability of a diet/an additive to reduce the infection or its consequences.

Yeast and health status

In vitro tests showed that *Saccharomyces cerevisiae* reduces the pathogenicity of *Escherichia coli* F4ac (ETEC) (Zanello *et al*., 2011. PLoS ONE 6(4): e18573).
In vivo, few data is available but favorable evidences are available (Trckova *et al*., 2014. J. Anim. Sci. 92:767–774).

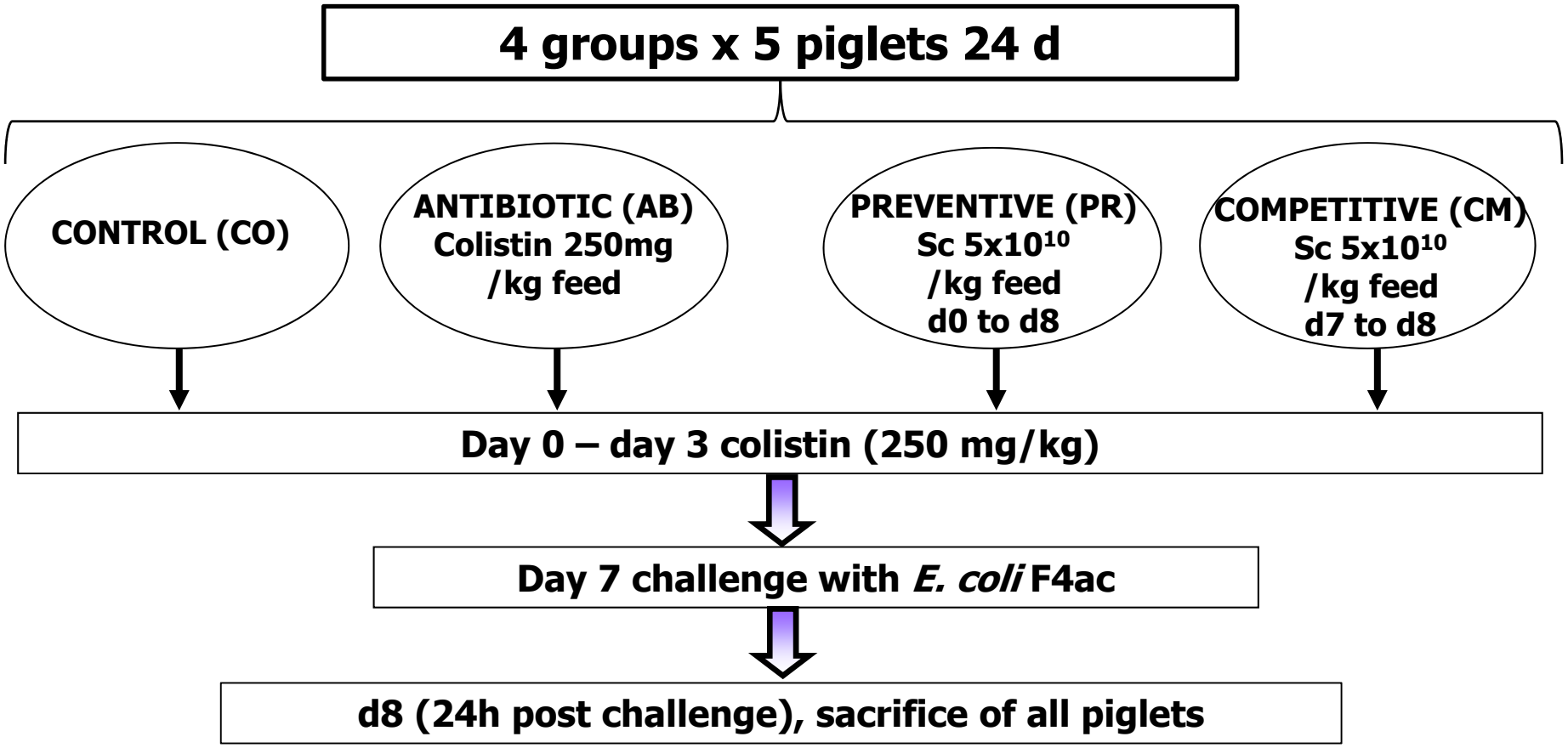
AIMS

Evaluate the effect of *Saccharomyces cerevisiae* CNCM I-4407 (**Sc**) supplied at different patterns on the transcriptome profile of the jejunum mucosa of pigs 24h after the infection with ETEC

Why 24 h from ETEC infection?

Before diarrhea appearance, to disclose the ability of live yeast to early modulate the cell signals in response to the infection.

EXPERIMENTAL DESIGN



RESULTS

All the pigs presented the specific receptors for the adhesion of *Escherichia* coli F4ac on the intestinal villi

(*in vitro* test)

GROWTH PERFORMANCE

- Body Weight **ns**
- Average daily live weight gain **ns**
- Average daily feed intake **ns**
- Gain to feed **ns**

INFLAMMATORY MARKER IN BLOOD 24 HOURS AFTER CHALLENGE

- CRP (mg/ml) ns
- Haptoglobin (mg/ml) ns

→ In the present conditions (24h p.i.), systemic inflammatory response was not yet mounted

→ No watery diarrhea occurred before sacrifice

INTESTINAL CELL RENEWAL ESTIMATION

	Diet					AB	YEAST	PR
	CO	AB	PR	CM	SME	*vs.* CO	*vs.* CO	*vs.* CM
Mitotic index (number of cell)								
- Villus	16.5	28.2	28.9	19.6	3.7	<0.05	ns	0.10
- Crypt	37.7	47.5	50.6	39.4	4.3	ns	ns	0.08
Apoptotic index (number of cell)								
- Villus	4.2	3.9	3.6	7.2	3.7	ns	ns	0.07
Zonula-1 (integrity score)								
- Villus	2.2	2.4	2.2	1.9	3.7	ns	ns	ns

→ **AB and PR treatments revealed trophic effects on gut mucosa (AB > PR)**

Gene Set Enrichment Analysis
AB vs CO

Enrichment in phenotype: AB (5 samples)
586 / 904 gene sets are upregulated in phenotype **AB**
143 gene sets are significant at FDR < 25%

NAME	FDR q-val
Genes sets enriched in AB group	
Regulation_of_mitosis	**0**
M_phase_of_mitotic_cell_cycle	**0**
Mitosis	**0**
Mitochondrial_membrane	**0**
Mitochondrial_inner_membrane	**0**

Enrichment in phenotype: CO (5 samples)
318 / 904 gene sets are upregulated in phenotype **CO**
17 gene sets are significantly enriched at FDR < 25%

NAME	FDR q-val
Genes sets enriched in CO group	
Cell_junction	**0.15**
Intercellular_junction	**0.16**
Chloride_channel_activity	**0.17**
Anion_channel_activity	**0.18**
Basolateral_plasma_membrane	**0.09**

Gene Set Enrichment Analysis
PR vs CO

Enrichment in phenotype: PR (5 samples)
599 / 904 gene sets are upregulated in phenotype **PR**
209 gene sets are significant at FDR < 25%

NAME	FDR q-val
Genes sets enriched in PR group	
Nuclear_part	**0.01**
Nuclear_pore	**0.01**
Mitotic_cell_cycle	**0.01**
Cell_cycle_phase	**0.00**
T_cell_activation	**0.03**

Enrichment in phenotype: CO (5 samples)
305 / 904 gene sets are upregulated in phenotype **CO**
10 gene sets are significantly enriched at FDR < 25%

NAME	FDR q-val
Genes sets enriched in CO group	
Structural_constituent_of_ribosome	**0.01**
Apicolateral_plasma_membrane	**0.26**
Apical_junction_complex	**0.20**
Anion_transmembrane_transporter_activity	**0.17**
Anion_channel_activity	**0.19**

Gene Set Enrichment Analysis
CM vs CO

Enrichment in phenotype: CM (5 samples)
646 / 904 gene sets are upregulated in phenotype **CM**
118 gene sets are significant at FDR < 25%

NAME	FDR q-val
Genes sets enriched in CM gropup	
Nucleobasenucleoside_and_nucleotide_metabolic_process	**0.14**
Cofactor_metabolic_process	**0.18**
Ligand_dependent_nuclear_receptor_activity	**0.23**
Oxidoreductase_activity	**0.22**
Response_to_oxidative_stress	**0.21**

Enrichment in phenotype: CO (5 samples)
258 / 904 gene sets are upregulated in phenotype **CO**
4 gene sets are significantly enriched at FDR < 25%

NAME	FDR q-val
Genes sets enriched in CO group	
Structural_constituent_of_ribosome	**0**
Structural_molecule_activity	**0.10**
Centrosome	**0.10**
Microtubule_organizing_center	**0.18**

ANOVA genes analyses
AB vs CO

Gene	Fold Change	p-value (AB vs. CO)	Full name	Biological function
Genes up-regulated in AB group				
APOC3	2.12	0.03	Apolipoprotein C-III	Synthesis of lipids
SCD	2.16	<0.01	Stearoyl-CoA desaturase	Cell growth and differentiation
SEC14L2	3.07	0.04	SEC14-like protein 2	Lipid-binding proteins
CA12	3.01	0.04	C	Catalyse the reversible hydration of carbon dioxide
GPT2	2.7	0.01	g a	Arginine metabolism
DNASE1L3	2.95	0.04	Deoxyribonuclease I-Like 3	DNA breakdown during apoptosis
ENPP7	3.6	0.02	Ectonucleotide pyrophosphatase/phosphodiesterase 7	digests sphingomyelin
Genes down-regulated in AB group				
GCNT3	-2.02	0.02	Glucosaminyl (N-Acetyl) Transferase 3, Mucin Type	Mucin biosynthesis
DUOX2	-3.53	0.02	Dual oxidase 2	Activation of bactericidal molecule
CCL20	-3.05	0.03	Chemokine (C-C Motif) Ligand 20	Response to infection by various enteropathogenic bacteria or exposure to bacterial flagellin
GPR126	-2.09	0.05	G-protein coupled receptor 126	Up-regulated by bacterial lipopolysaccharides (LPS).

Cell energy utilization

⬇

GUT HEALTH

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CA12	3.01	0.04	Carbonic anhydrases 12	Catalyse the reversible hydration of carbon dioxide
GPT2	2.7	0.01	glutamic pyruvate transaminase (alanine aminotransferase) 2	Arginine metabolism
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Genes involved in the response against pathogens

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Not necessary with Antibiotic

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GPR126	-2.09	0.05	G-protein coupled receptor 126	Up-regulated by bacterial lipopolysaccharides (LPS).

Down-regulated by the LPS infection in a cell culture system. Hohenhausa *et al.* (2013). Immunobiology 218: 1345–1353

ANOVA genes analyses
PR vs CO

Gene	Fold Change	p-value (PR vs. CO)	Full name	Biological function
Genes up-regulated in PR group				
GZMK	1.84	0.04	Granzyme K	Innate immune response
PRKCQ	1.82	<0.01	Protein kinase C theta type	cellular signalling pathways
IKZF3	1.68	0.03	Ikaros family zinc finger protein 3	B lymphocyte proliferation and differentiation
RASGRP1	1.62	0.03		
CD8A	1.6	0.02		s to recognize
IKZF1	1.54	0.02	Ikaros family zinc finger protein 1	lymphocyte differentiation.
Genes down-regulated in PR group				
IFRD1	-1.57	0.001	Interferon-related developmental regulator 1	cell differentiation
VEGFA	-1.62	0.02	Vascular Endothelial Growth Factor A	cell growth/promoting cell migration/inhibiting apoptosis
TFF3	-1.79	0.03	Trefoil factor 3	stabilize the mucus layer/affects healing of the epithelium
CCL20	-3.02	0.04	Chemokine (C-C Motif) Ligand 20	Bacterial recognition

Differentiation and proliferation of lymphocytes T and B

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ETEC stimulated the canonical NFκB pathways

ANOVA genes analyses
PR vs CO

Gene	Fold Change	p-value (PR vs. CO)	Full name	Biological function
Genes down-regulated in PR group				
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VEGFA	-1.62	0.02	Vascular Endothelial Growth Factor A	ration/inhibiting apoptosis
TFF3	-1.79	0.03		healing of the
CCL20	-3.02	0.04	Chemokine (C-C Motif) Ligand 20	Bacterial recognition

IFN-inducible gene, mainly by LPS
(Mulder *et al.* - BMC Biology .2009, 7:79)

Target gene involved in the induction of the mucosal inflammation.
(Scaldaferri *et al.* - Gastroenterology. 2009, 136: 585–595)

Strongly induced after mucosal injury.
(Taupin *et al.*, - Lab. Invest. 2001, 81:397-408).

Up-regulated in a variety of inflammatory disorders.
(Sibartie *et al.* - BMC Immunol, 2009)

S. cerevisiae inhibits the ETEC-induced expression of CCL20 in IPEC-1 cell culture study.
(Zanello *et al.* - PLoS ONE 6(4): e18573, 2011)

Single genes analyses
CM vs CO

Gene	Fold Change	p-value (CM vs. CO)	Full name	Biological function
Genes up-regulated in CM group				
IGF1	2.02	0.03	Insulin-like growth factor 1	cell growth and proliferation
THOC5	1.52	0.03	THO complex subunit 5 homolog	cell differentiation processes
PPARGC-1	1.63	0.05	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	inhibits pro-inflammatory cytokine production
STEAP4	1.58	0.02		of inflammatory cytokines
UNC5CL	1.65	<0.001		flammation and immunity
SOX6	1.52	0.03	Transcription factor SOX-6	transcriptional activator
Genes down-regulated in CM group				
CENPK	-1.52	0.02	Centromere protein K	mitotic progression
TRPC1	-1.57	<0.01	Transient receptor potential channel 1	nonspecific cation channel
FGL2	-1.57	0.01	Fibrinogen-like protein 2	immunoregulatory - effector cytokine of Treg cells
SAMD9	-1.57	0.04	Sterile Alpha Motif Domain Containing 9	cellular proliferation
IL7R	-1.58	0.02	Interleukin 7 Receptor	survival factor for mature T cells and IEC
CPT1A	-1.64	0.01	Carnitine palmitoyltransferase I	mitochondrial enzyme
FABP1	-1.77	0.03	Fatty acid-binding protein 1	bind bile acids
PLIN2	-1.9	0.02	Perilipin 2	adipocyte differentiation
CD163	-2.47	0.04	(Cluster of Differentiation 163	induction of local inflammation

Intestinal mucosa homeostasis

ANOVA genes analyses
CM vs CO

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Genes up-regulated in CM group				
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PPARGC-1	1.63	0.05		
STEAP4	1.58	0.02	prostate 4	regulation of inflammatory cytokines
UNC5CL	1.65	<0.001	Unc-5 Homolog C (C. Elegans)-Like	epithelial inflammation and immunity
SOX6	1.52	0.03	Transcription factor SOX-6	transcriptional activator
Genes down-regulated in CM group				
CENPK	-1.52	0.02	Centromere protein K	mitotic progression
TRPC1	-1.57	<0.01	Transient receptor potential channel 1	nonspecific cation channel
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Expressed in mucosal epithelia, pro-inflammatory activity, identified as a putative candidate molecule causally involved in mucosal diseases such as IBD.
(Heinz *et al.* Cell Death Differ. 2012, 19: 722–731).

ANOVA genes analyses
CM vs CO

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UNC5CL	1.65	<0.001	Unc-5 Homolog C (C. Elegans)-Like	epithelial inflammation and immunity
SOX6	1.52	0.03	Transcription factor SOX-6	transcriptional activator
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CD163	-2.47	0.04	(Cluster of Differentiation 163	induction of local inflammation

Mucosal inflammation process

CONCLUSIONS

- Preventive administration of *Saccharomyces cerevisiae* CNCM I-4407, seems to limit the early activation of gene sets related with the impairment of the jejunum mucosa of piglets infected with ETEC.
- Competitive administration of *Saccharomyces cerevisiae* CNCM I-4407, partially reduce the detrimental effect of ETEC

Take home message:

$5x10^{10}$ CFU/kg feed of *Saccharomyces cerevisiae* CNCM I-4407, reduce the early cell signaling of inflammation in the GIT of ETEC susceptible weaned pigs

THANK YOU FOR YOUR ATTENTION!!!
& Have a safe trip back home

Contacts:
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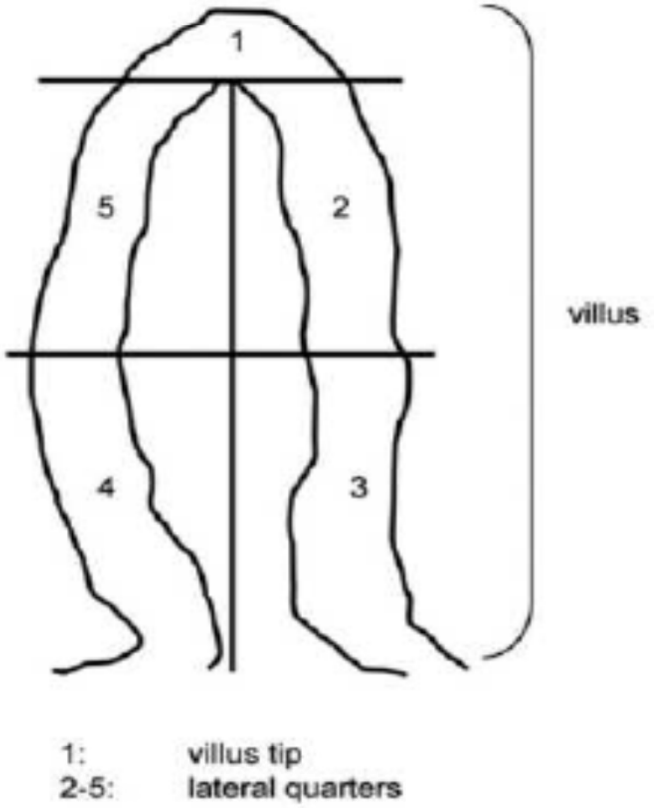
FECAL SCORE

	Diet							
Hours	CO	AB	PR	CM	**SEM**	**AB *vs.* CO**	**YEAST *vs.* CO**	**PR *vs.* CM**
- 12	2.0	1.5	2.0	2.2	0.37	ns	-	
12	2.9	2.1	1.9	2.4	0.32	0.09	0.09	ns
24	4.0	2.1	3.5	3.4	0.47	0.01	ns	ns

→ No watery diarrhea occurred before sacrifice

ZO-1 EXPRESSION ON VILLUS

Score 1=ZO-1 expression completely absent
Score 2= ZO-1 expression partially present
Score 3= ZO-1 expression completely present



Klunker *et al.* (2013)

Concentration of Sc in the diet used in each run

Sample Ref	Type	CFU/g	Log CFU/g
Swine feed sample 1	Mash	8,4E+07	7,92
Swine feed sample 2	Mash	9,3E+07	7,97
Swine feed sample 3	Mash	7,9E+07	7,90

Expected CFUs: $5x10^7$ CFU/g,

Sample Ref	Type	CFU/g	Log CFU/g
Swine feed sample 1	Mash	5,6E+07	7,75
Swine feed sample 2	Mash	5,4E+07	7,73
Swine feed sample 3	Mash	4,9E+07	7,69

Sample Ref	Type	CFU/g	Log CFU/g
Swine feed sample 1	Mash	1,9E+07	7,29
Swine feed sample 2	Mash	2,4E+07	7,39
Swine feed sample 3	Mash	1,7E+07	7,24

BACKGROUND

→ Predicting the susceptibility on live piglets?

- Susceptibility is associated with the presence of a genetic variant for the Mucin 4 gene (MUC4)
- A XbaI polymorphism of the porcine MUC4 used as a marker for the susceptibility of piglets

→ Lack of the F4ac receptors (and hence resistance to neonatal diarrhoea) is an autosomal recessive trait