Comparison between yeast administration at different timing on health status of weaned pigs in response to Escherichia coli F4ac infection

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Introduction

The enterotoxigenic *Escherichia coli* F4ac (ETEC) is one of the most diffuse causative agent responsible of post-weaning diarrhea (PWD) in pigs. Nowadays, reducing the deleterious effect of this pathogen without antibiotic use is one of the main goal and the yeast administration could represent a valid strategies.

Aims

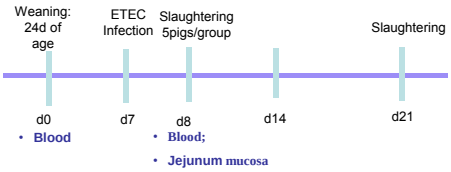
This study aims to disclose the application of *Saccharomyces cerevisiae* CNCM I-4407 (Sc), supplied at different patterns, to contain the detrimental effect of ETEC in weaned pigs.

Material and methods

Animals and sampling

Sixty piglets weaned at 24 days of age (d0) were allotted to one of following groups for 21 days:

- Control (CO)
- CO+colistin, 1g/kg feed (AB),
- CO+5x10^{10} CFU of Sc/kg feed, from d0 to d21 (PR),
- CO+5x10^{10} CFU of Sc/kg feed from d7 (infection with ETEC) to d11 (CM).



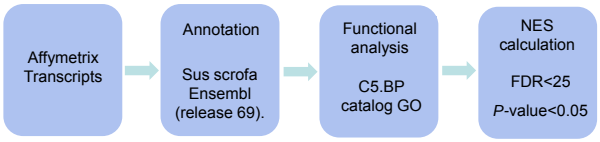
1. Fecal scores: 1 to 5: where 1 = hard, 5 = watery feces.

2. Metabolomics: Blood serum was analysed using AbsoluteIDQ p180 Kit (Biocrates Life Science AG, Innsbruck, Austria)

3. Transcriptomic: Jejunum transcript was carried out using Affymetrix©Porcine Gene 1.1 ST array strips.

Statistical analysis

- Performance and faecal score data were analyzed by ANOVA with a completely randomized design, 2 blocks (time), sows within block, and 4 dietary treatment.
- The metabolomics data were analysed on SAS software (SAS Inst., Inc., Cary, NC) using linear mixed models including a random effect for litter. Results were validated using permutation test and LOO procedure



Results

1) Effect of *Saccharomyces cerevisiae* on the faecal score

	Diet					Contrast		
Hours	CO	AB	PR	CM	SEM	AB vs. CO	PR+CM vs. CO	PR vs. CM
−122	2.4	2	2.1	1.9	0.2	0.89	0.773	
12	2.9	2.2	2.5	2.3	0.2	0.02	0.044	0.51
24	3.7	2.4	3	3.2	0.3	0.001	0.084	0.57
48	4.2	2.4	3.4	3.7	0.3	<0.001	0.04	
72	4.1	2.6	3.4	3.9	0.4	<0.05	0.41	
144	3	1.9	3.2	3.3	0.3	0.02	0.73	

The yeast administration (PR+CM) compared with the CO group, reduced the severity of diarrhea until 48 hours after infection.

3) Transcriptomics: UP regulated and down regulated genes

Gene	Fold Change	p-value (AB vs. CO)	Full name	Biological function
Genes up-regulated in AB group				
GPT2	2.7	0.01	Glutamic pyruvate transaminase	Arginine metabolism
SCD	2.16	<0.01	Stearoyl-CoA desaturase	Cell growth and differentiation
APOC3	2.12	0.03	Apolipoprotein C-III	Synthesis of lipids
Genes down-regulated in PR 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

Gene	Fold Change	p-value (PR vs. CO)	Full name	Biological function
Genes up-regulated in PRgroup				
GZMK	1.84	0.04	Granzyme K	Innate immune response
PRKCQ	1.82	<0.01	Protein kinase C theta type	cellular signalling pathways
Genes down-regulated in PR group				
VEGFA	-1.62	0.02	Vascular Endothelial Growth Factor A	migration/inhibiting apoptosis
TFF3	-1.79	0.03	Trefoil factor 3	stabilize the mucus layer
CCL20	-3.02	0.04	Chemokine (C-C Motif) Ligand 20	Bacterial recognition

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	1.63	0.05	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	inhibits pro-inflammatory cytokine production
Genes down-regulated in CM group				
TRPC1	-1.57	<0.01	Transient receptor potential channel 1	nonspecific cation channel
FGL2	-1.57	0.01	Fibrinogen-like protein 2	effector cytokine of Treg cells

2) Metabolomics: Effect of *Saccharomyces cerevisiae* on blood metabolic profile

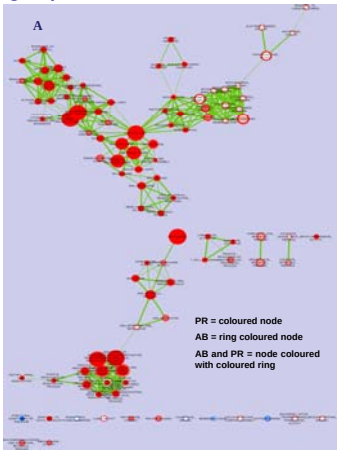
Diet1/metabolites	P-value	Direction
AB vs. CO		
C12:1[2]	<0.01	CO
C5DC(C6-OH)[3]	0.02	CO
PC_aa_C40:1[4]	0.01	CO
PC_aa_C40:6[5]	<0.01	CO
alpha-AAA[6,7]	<0.01	CO
CM + PR vs.CO		
SM_C18:0[8,7]	0.02	CM + PR ↓
C10:2[9]	0.02	CM + PR
CM vs. PR		
C10:2	<0.01	CM

2Dodecenoyl-L-carnitine. 3Glutaryl-L-carnitine/hydroxyhexanoyl-L-carnitine. 4Phosphatidylcholine diacyl C 40:1. 5Phosphatidylcholine diacyl C 40:6. 6Alpha-amino adipic acid. 7Affected by the confounding factor "fecal score." 8Sphingomyelin-ceramide. 9Decadienyl-L-carnitine

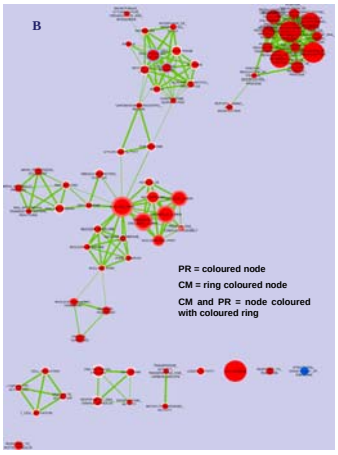
The *Saccharomyces cerevisiae* administration (CM+PR vs. CO) 24 h after infection with ETEC significantly reduced the level of Sphingomyelin-ceramide and increased the concentration of Decadienyl-L-carnitine two compounds involved in acute inflammation and anti inflammatory pathway

3) Transcriptomics: Gene set enrichment analysis (A and B)

PR and AB groups compared to CO group



PR and CM groups compared to CO group



Enrichment significance (-value) is conveyed as node colour intensity, where red stands for upregulation, and blue for downregulation with PR or CO treatments, and white for no effect of the treatments. The size of the nodes represents how many genes are in the gene set.

Conclusion

- The prophylactic use of *Sc* CNCM I-4407 is able to prevent the detrimental effect of ETEC infection in susceptible pigs, this is confirmed by the gene expression profile 24h after the challenge.
- The competitive administration of the yeast slightly reduce the negative effect of ETEC infection, partially maintaining some metabolic processes and reducing the impact of post-challenge inflammation.
- The competitive administration of *Sc* CNCM I-4407 offers the perspective to develop a new generation of probiotics, already effective shortly after the infection, for a most targeted use against the pathogen in the post-antibiotic era.