Interplay Workshop 29 August 2013 – EAAP - Nantes

Effects of early microbiota association and dietary interventions on gut development and function in piglets

A.J.M. Jansman, S.J. Koopmans, J. Zhang and H. Smidt

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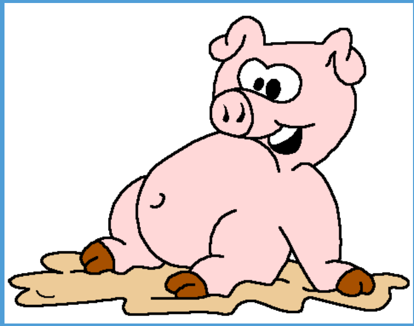
Introduction

- Intestinal health of pigs had large focus over the past 10 years
- Focus on weaning and gut health in the post weaning period
- Use of dietary interventions (nutrients, ingredients and feed additives)
- Low attention to the role and importance of the immediate postnatal colonisation of the gut and long term consequences for gut health and performance



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Intestinal microbiota and long term health effects

- Effects on the functional development of the gut (barrier function)
- Effects on development of the immune system and development of allergies and auto immune diseases
- Effects on the development of obesity
- Influence on the predisposition for inflammatory bowel disease (IBD)
- Effects of early antibiotic treatment

Long term effects of intestinal microbiota on animal health and performance

Recent papers

The ISME Journal (2008) 2, 739–748
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www.nature.com/ismej

ORIGINAL ARTICLE

The immediate environment during postnatal development has long-term impact on gut community structure in pigs

Claire L Thompson, Bing Wang and Andrew J Holmes
School of Molecular and Microbial Biosciences, University of Sydney, Sydney, New South Wales, Australia

REVIEWS

The gut microbiota shapes intestinal immune responses during health and disease

June L. Round and Sarkis K. Mazmanian

Abstract | Immunological dysregulation is the cause of many non-infectious human diseases such as autoimmunity, allergy and cancer. The gastrointestinal tract is the primary site of interaction between the host immune system and microorganisms, both symbiotic and pathogenic. In this Review we discuss findings indicating that development ... of the adaptive immune system are influenced by bacterial colonization of the ... highlight the molecular pathways that mediate host–symbiont interaction ... proper immune function. Finally, we present recent evidence to support ... in the bacterial microbiota result in dysregulation of adaptive immune ... underlie disorders such as inflammatory bowel disease. This raises the ... the mammalian immune system, which seems to be designed to cont... is in fact controlled by microorganisms.

Original Research Article

Developmental Origins of Obe...ty: Early Feeding E... Infant Growth, and the Intestinal Microbiome

AMANDA L. THOMPSON[1,2]*
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[2]Carolina Population Center, University of North Carolina ...

...ERICAN JOURNAL OF HUMAN BIOL...

Modulation of Systemic Immune Responses through Commensal Gastrointestinal Microbiota

Kyle M. Schachtschneider[1], Carl J. Yeoman[2,3], Richard E. Isaacson[4], Bryan A. White[1,2], Lawrence B. Schook[1,2]*, Maria Pieters[2]

...ogy

ORIGINAL ARTICLE

Direct experimental evidence that early-life farm environment influences regulation of immune responses

Marie C. Lewis[1]*, Charlotte F. Inman[1]*, Dilip Patel[1], Bettina Schmidt[2], Imke Mulder[2], Bevis Miller[1], Bhupinder P. Gill[3], John Pluske[4], Denise Kelly[2], Christopher R. Stokes[1] & Michael Bailey[1]

[1]Infection and Immunity, School of Veterinary Science, University of Bristol, Langford, Somerset, UK; [2]Gut Immunology Group, University of Aberdeen, Rowett Institute, Aberdeen, UK; [3]DEFRA, London, UK; [4]School of Veterinary and Biomedical Sciences, Murdoch University, Perth, Western Australia

ELSEVIER
Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Pharmacology

Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota

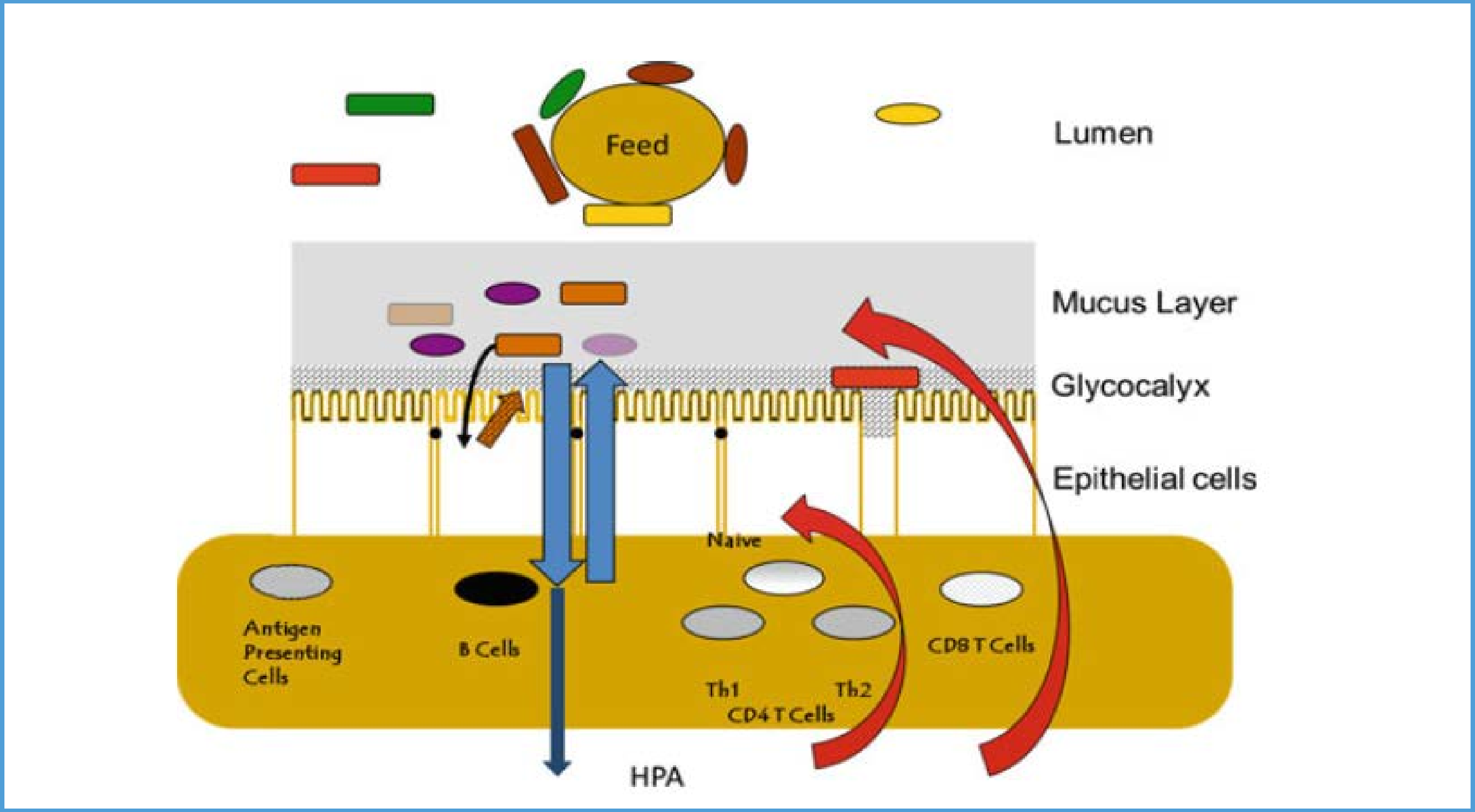
Patrice D Cani and Nathalie M Delzenne

ELSEVIER
Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Biotechnology

Programming infant gut microbiota: influence of dietary and environmental factors

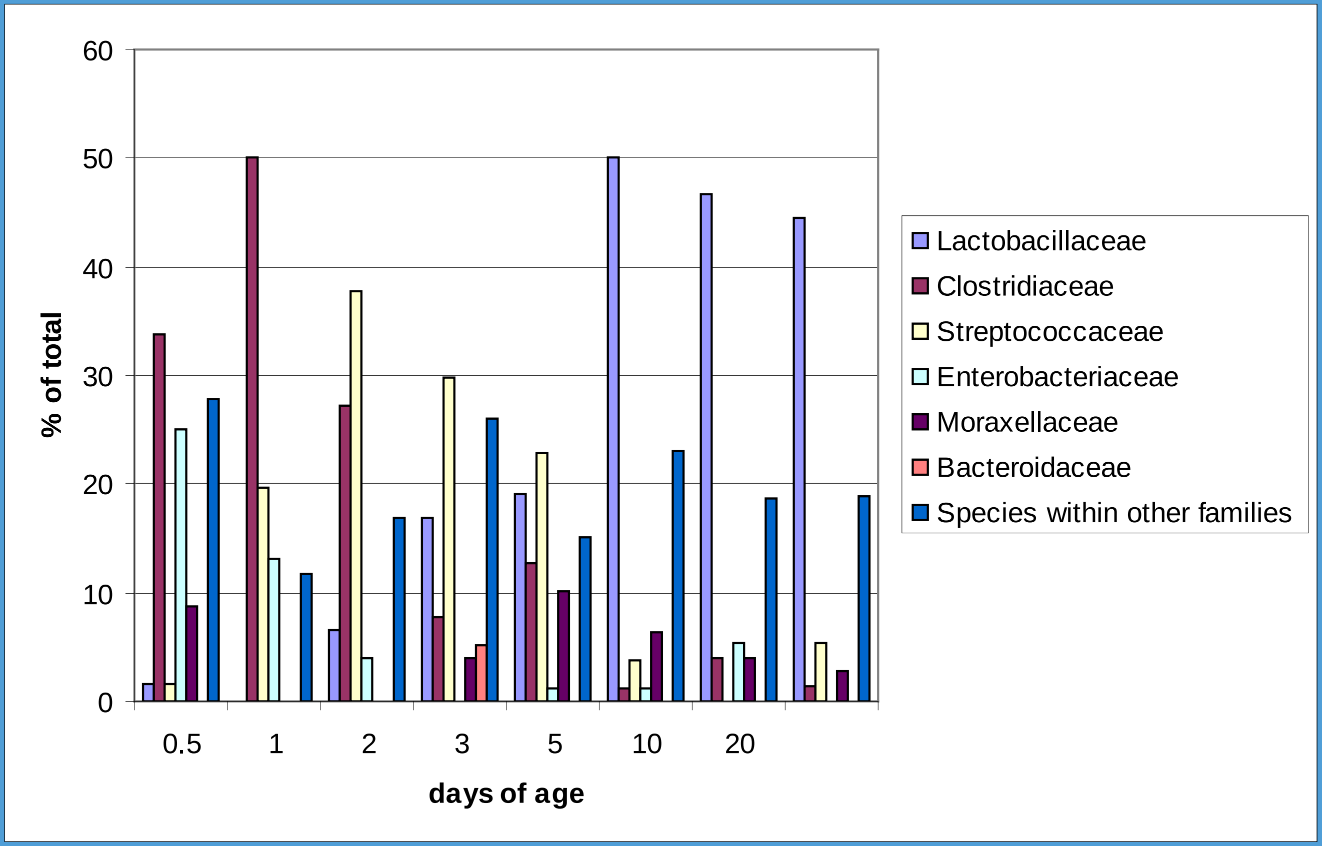
Tatiana Milena Marques[1,2,3], Rebecca Wall[1], R Paul Ross[1,2], Gerald F Fitzgerald[1,3], C Anthony Ryan[4] and Catherine Stanton[1,2]

The interaction between microbiota and the host at intestinal level



Patterson, 2012

Microbial succession in the digestive tract of piglets after birth



In total 604 species identified using ^{16}S rRNA gene sequencing

Petri et al., 2010

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Outdoor, Indoor and Isolator Environments

Kelly et al., 2010

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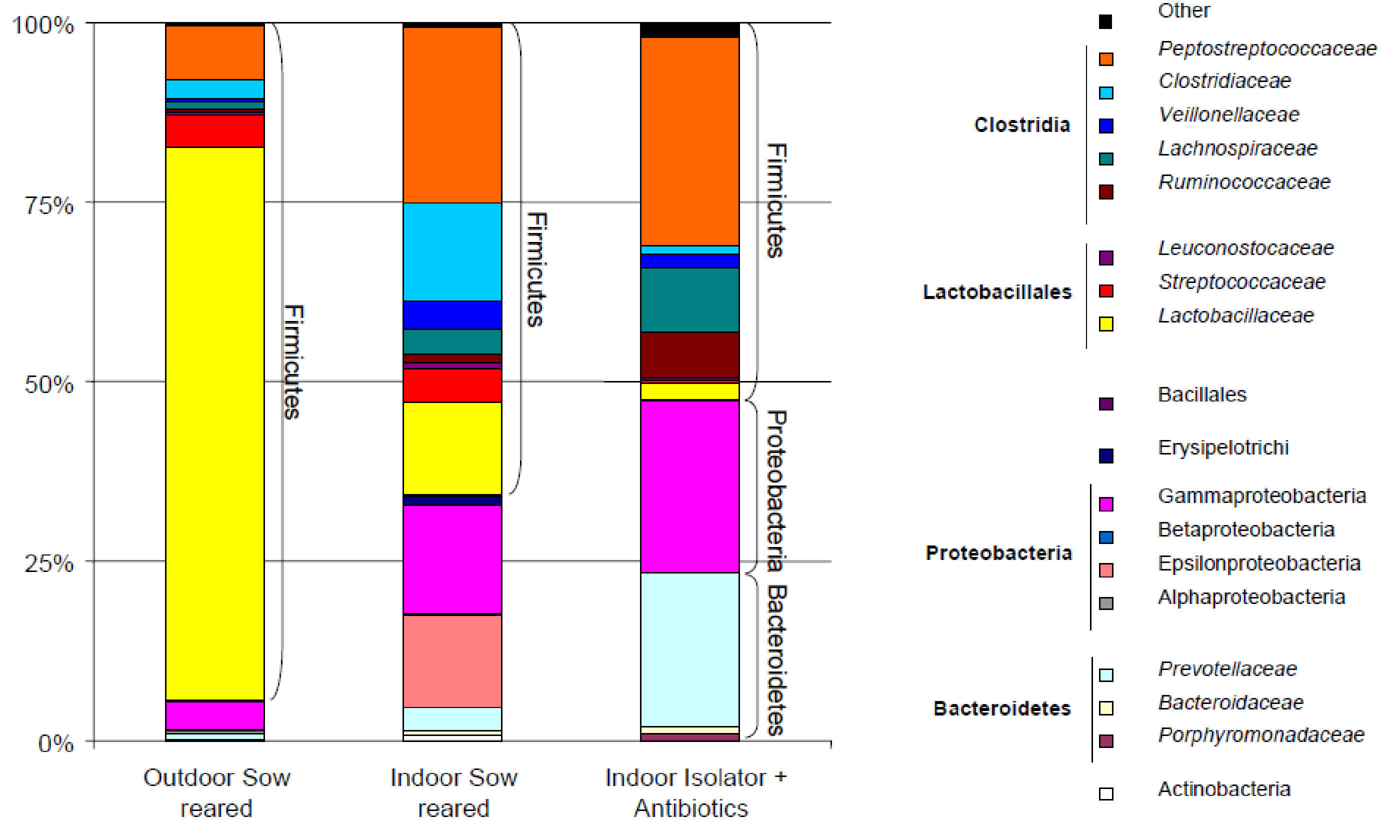
Early-life Environment Significantly Alters Microbial Mucosa-Associated Diversity



Phylogenetic Distribution of 16S rDNA Sequences

Mulder et al. 2009 BMC Biology

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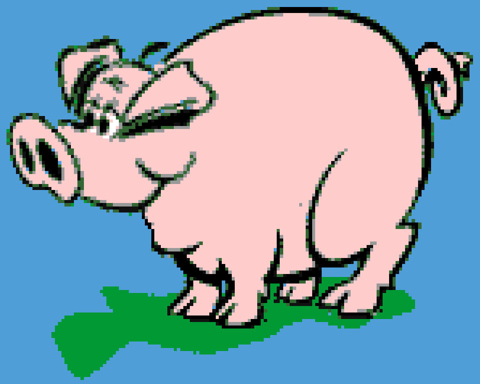
Mulder et al., 2009

Studies in the Interplay project

The first phase was designed to develop a model in piglets that allows the investigation of the effects of postnatal association with a simple or a complex microbiota on gut health and development.



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Material & methods - CDCD piglets

- Two SPF sows delivered 30 CD-piglets (13 and 17 resp.).
- 30 piglets subdivided into two groups in two “clean” SPF rooms (15 per pen).
- All piglets were orally dosed with a fixed volume of blood serum (supply of immunoglobulins).
- At the end of the day, all piglets were hand-fed a fixed amount of serum and milk for SPF piglets.
- From day 1 to 3, all piglets received an inoculum with a mixture of three bacterial species (“Bristol mix” consisting of Lactobacillus amylovorus (3.6 x 10^7 cfu), Clostridium glycolicum (5.7 x 10^7 cfu) and Parabacteroides sp. (4.8 x 10^7 cfu) as a means to standardize the intestinal microflora during the first days of life.

Treatments

From day 3 onwards each piglet were subjected to one of the two experimental treatments:

- Treatment 1 (SA)(n=15), association with three microbial species (d 1-3), SPF conditions
- Treatment 2 (CA) (n=15), association with three microbial species (d 1-3) and orally receiving a fixed amount of diluted faeces (2 ml of an inoculant consisting of 10% saline diluted faeces) via oral gavage from a conventional sow (treatment imposed during day 3 and 4 of the study), SPF conditions.

CDCD piglets

- Piglets were fed the milk based diet for a period of 3 days (day 0-3 of the study) (4 feedings per day) using a milk-boot
- On day 4 after birth, the milk diet for both groups of piglets was replaced by a liquid diet for both experimental groups. The liquid feed was provided by an automated feeding-system.
- Throughout study faeces samples were collected and on d 28, piglets of both groups were euthanized for collection of blood, digesta and various tissue samples.

Caesarean delivery

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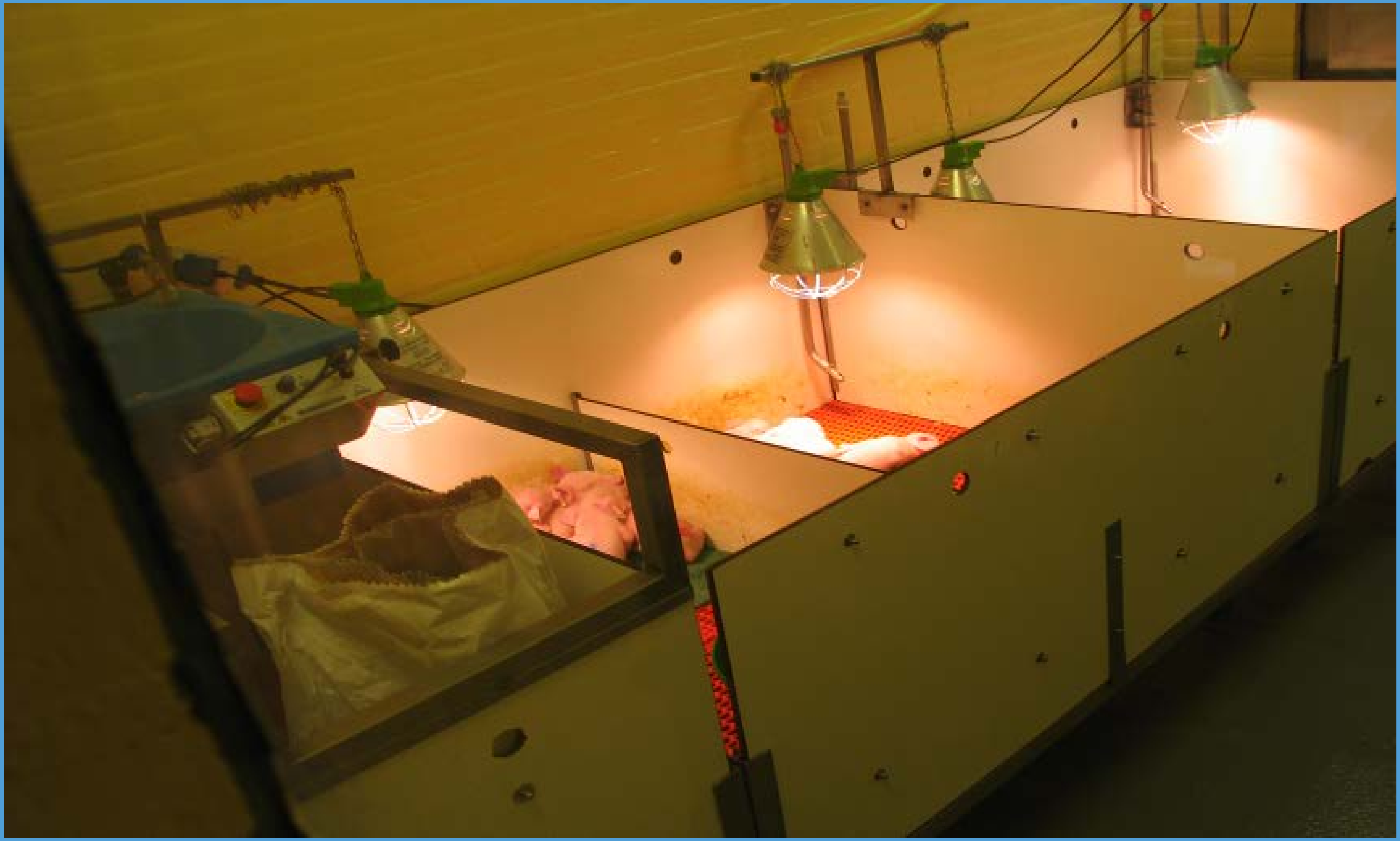
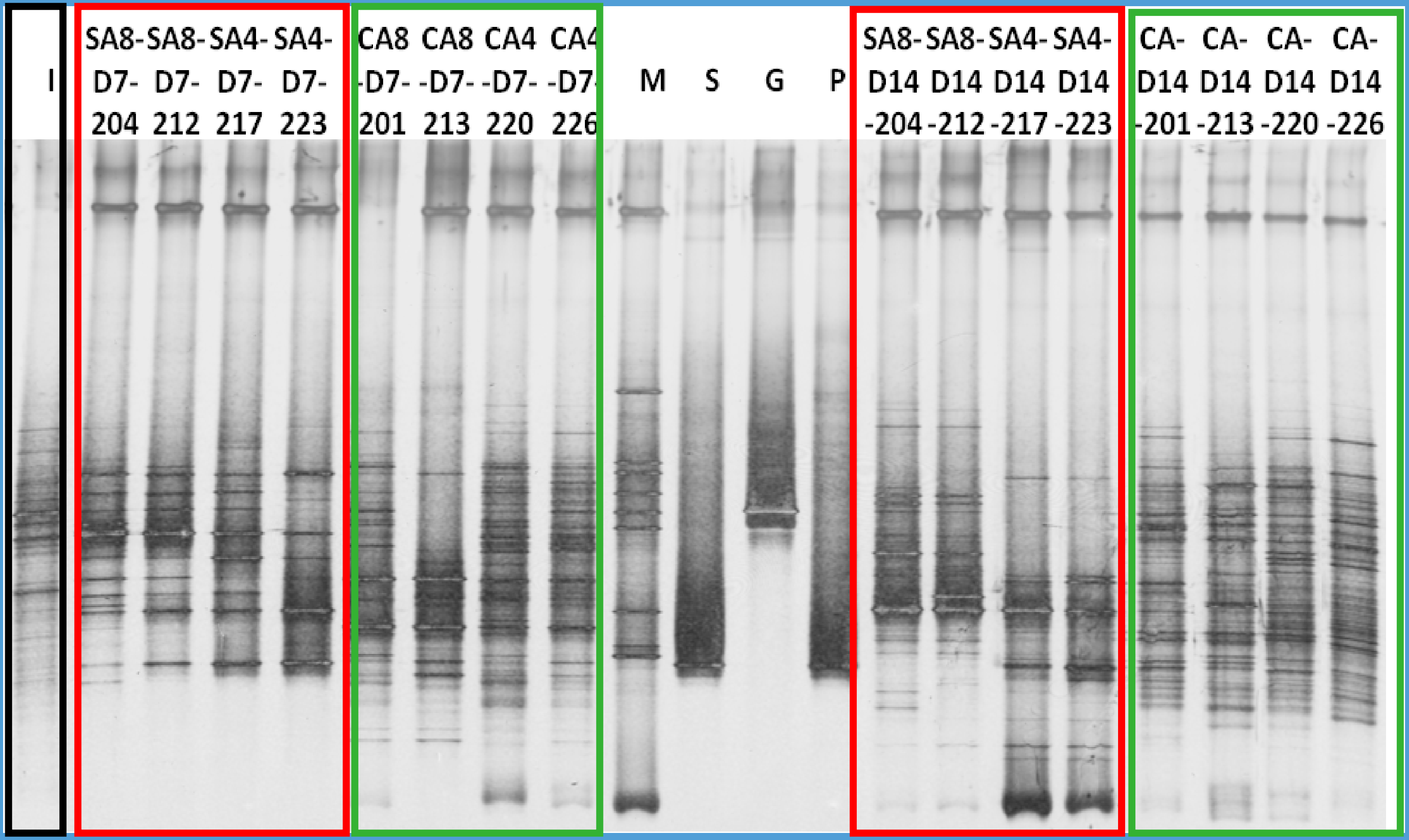


DGGE analysis d 7 and 14



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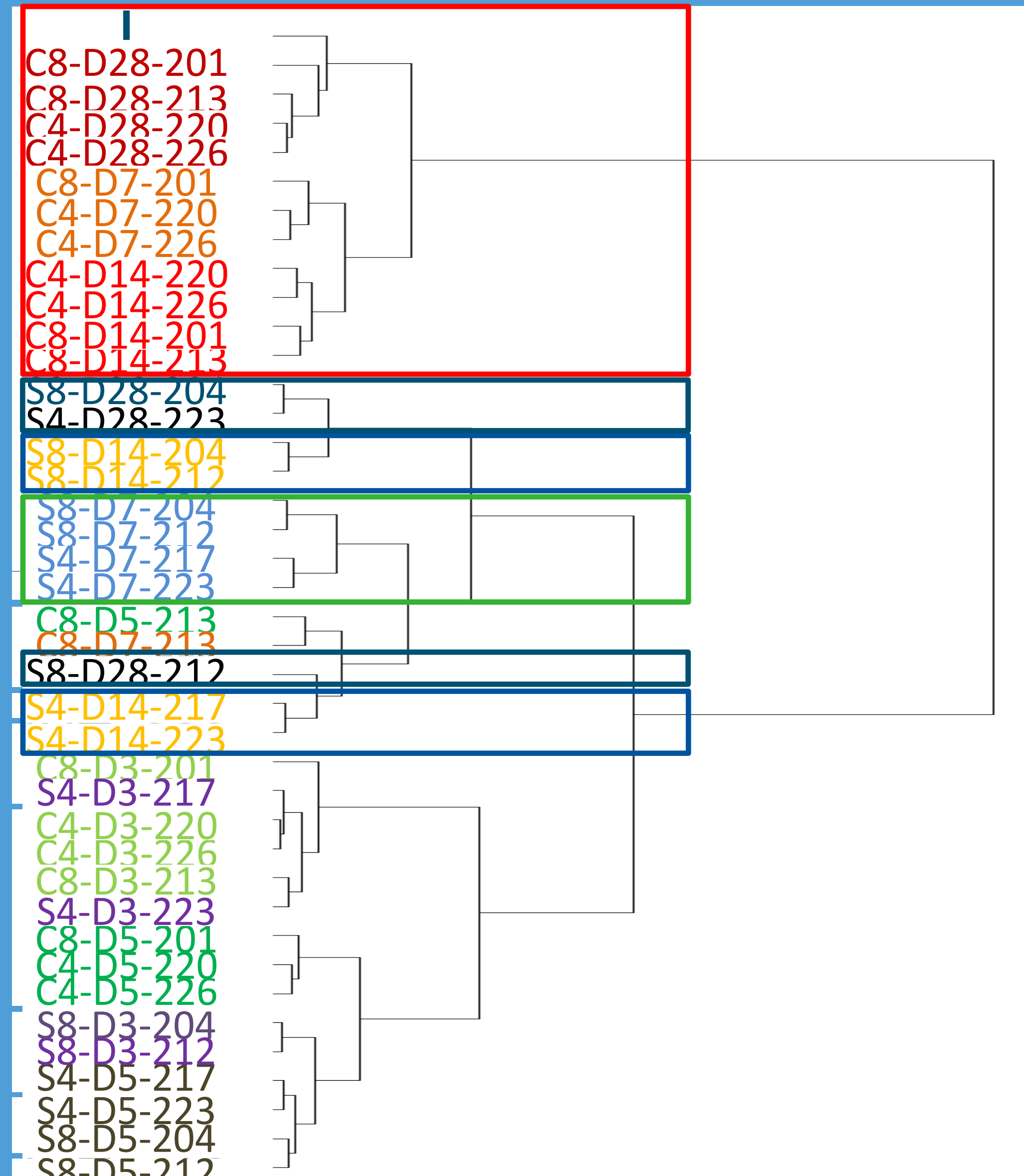
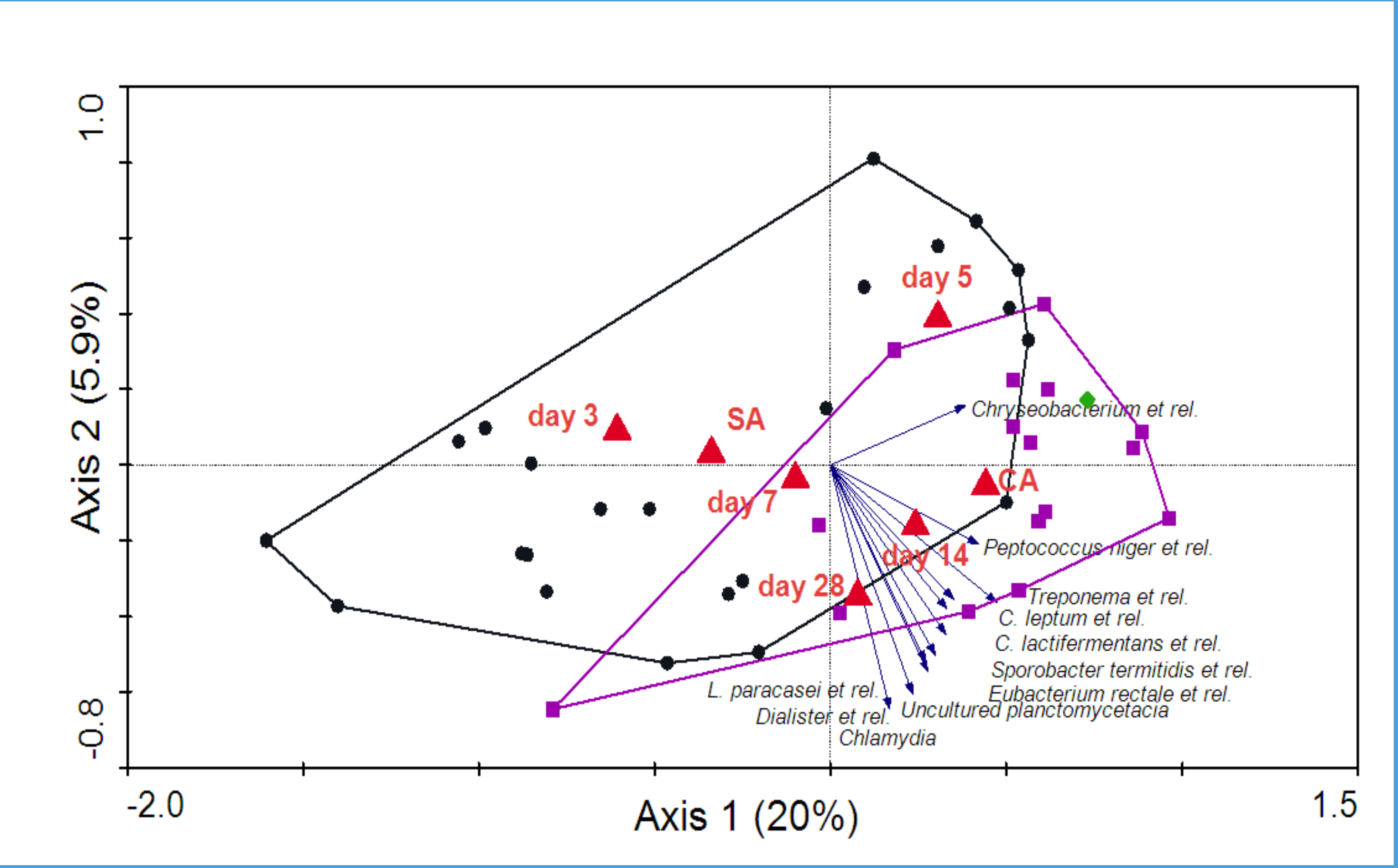
PITChip results

Cluster analysis

Day 28 CA Treatment
Day 14 CA Treatment
Day 7 CA Treatment
Day 5 CA Treatment
Day 3 CA Treatment
Day 28 SA Treatment
Day 14 SA Treatment
Day 7 SA Treatment
Day 5 SA Treatment
Day 3 SA Treatment

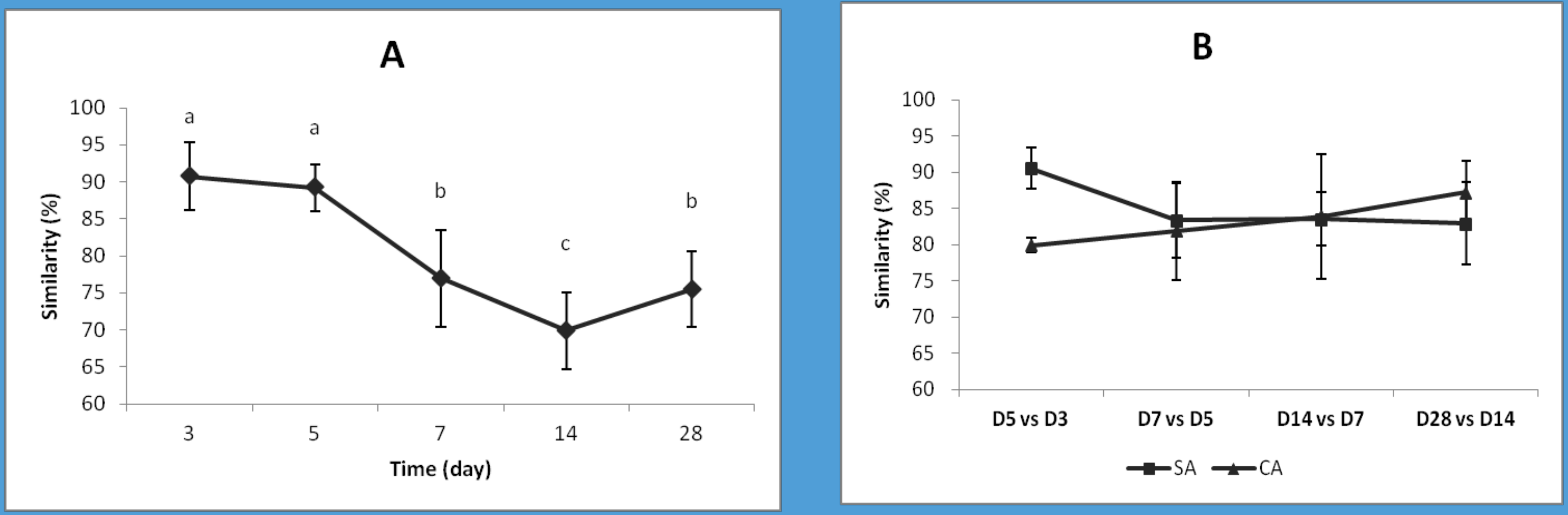


Comparison of bacterial communities among treatments



P treatment: 0.002

Similarity of bacterial communities in faecal samples as affected by treatment (B) and time of sampling (A)



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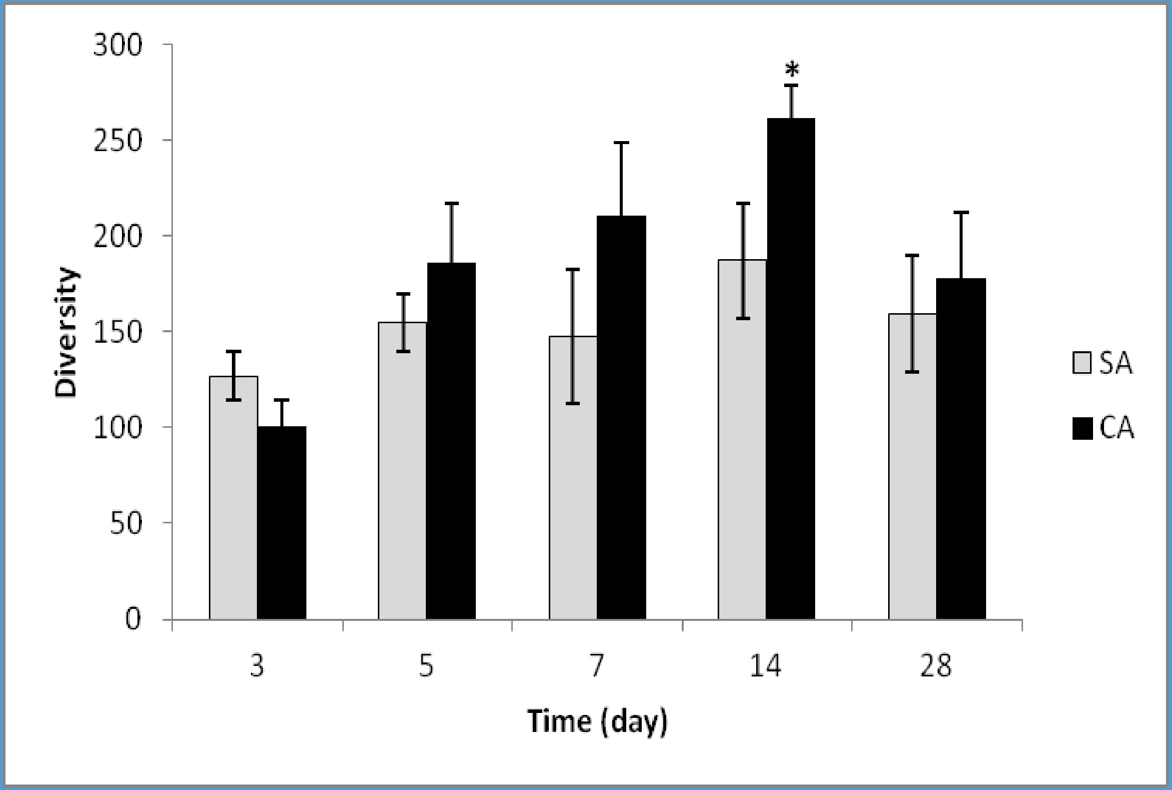
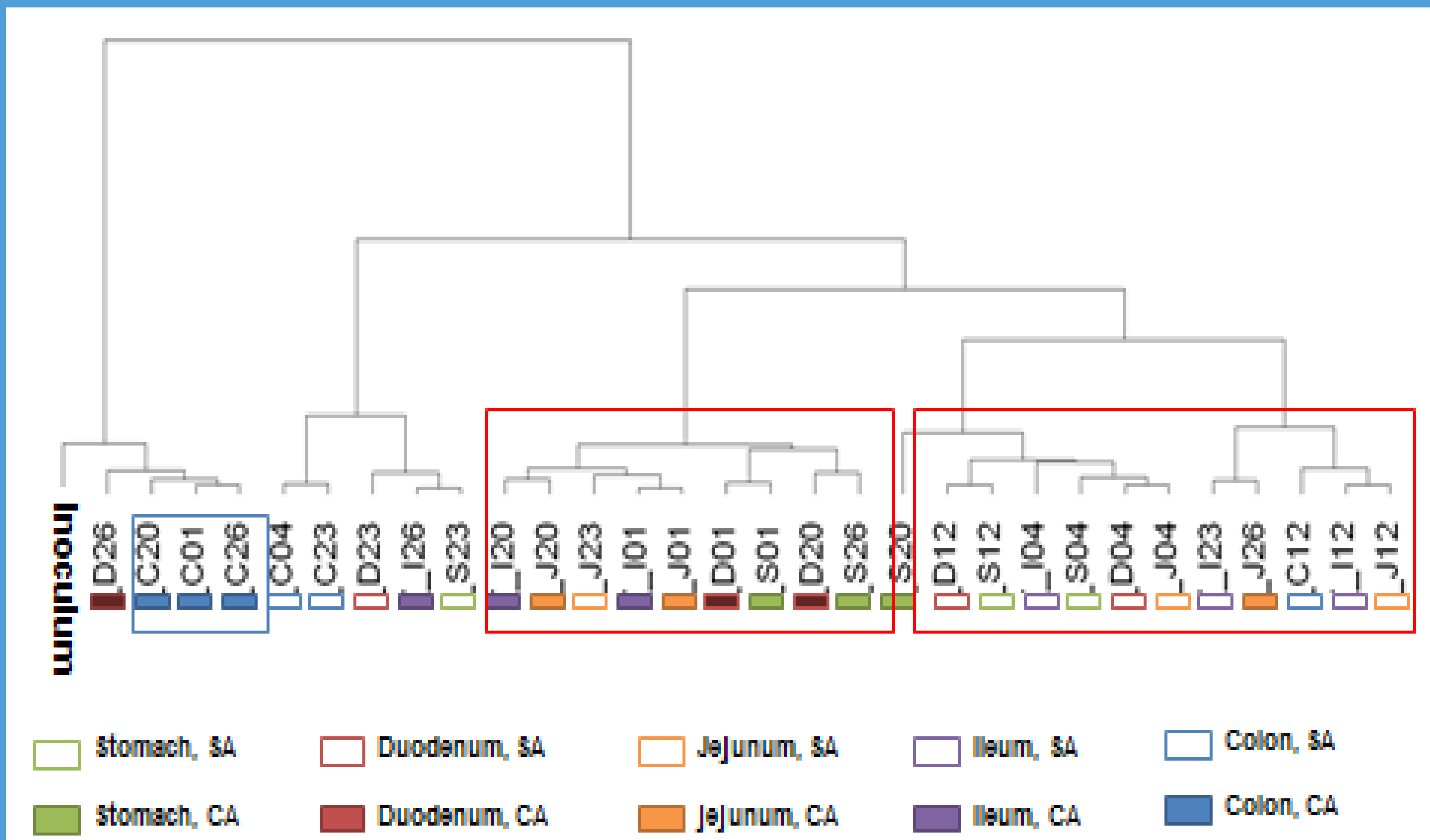
Diversity of bacterial communities in faeces among treatments

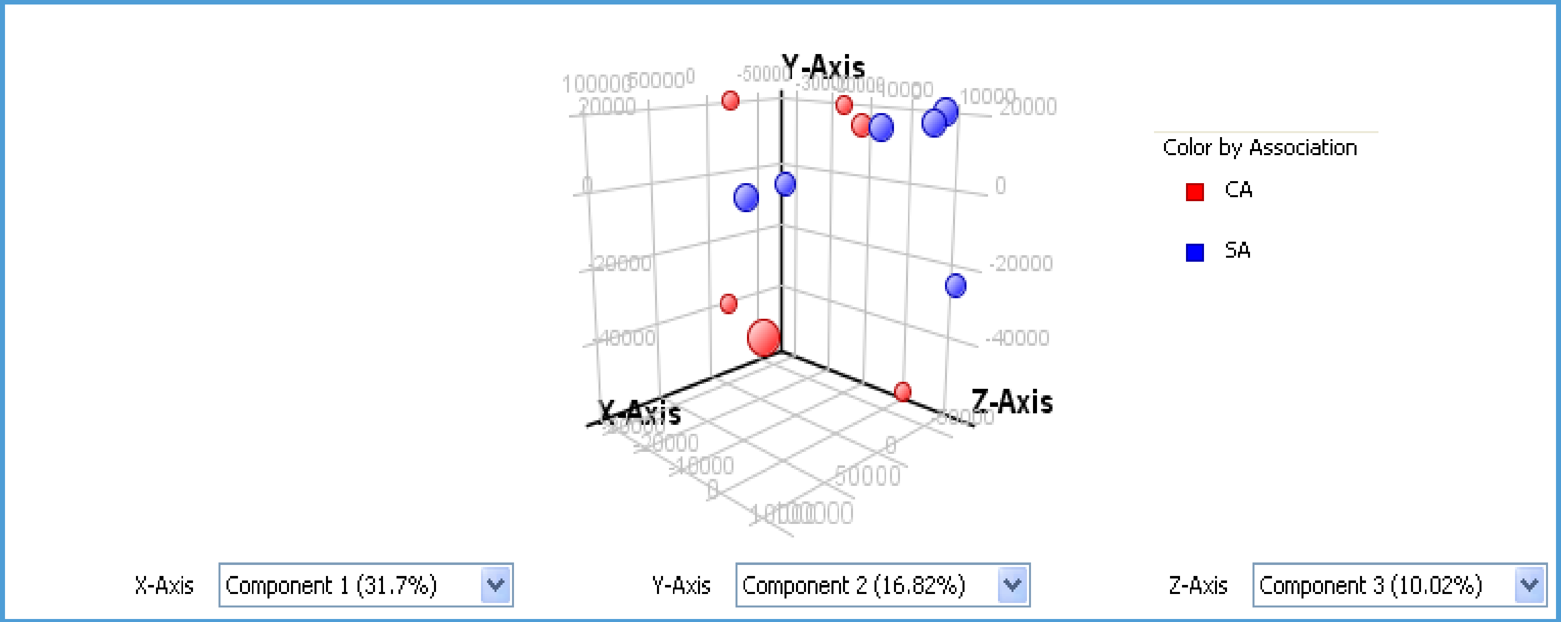


Diversity of bacterial communities in faecal samples as affected by treatment of piglets (simple association (SA) and complex association (CA)) and by time of sampling.
* indicates significant difference between treatments (P<0.01).

Comparison of microbiota communities in stomach, duodenum, jejunum, ileum and colon samples from d 28 using PIT chip analysis



PCA analysis gene expression ileal mucosa d 16



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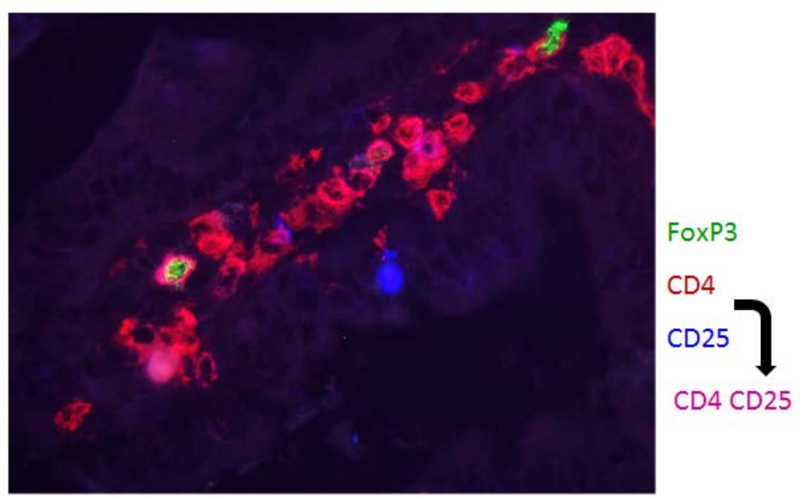
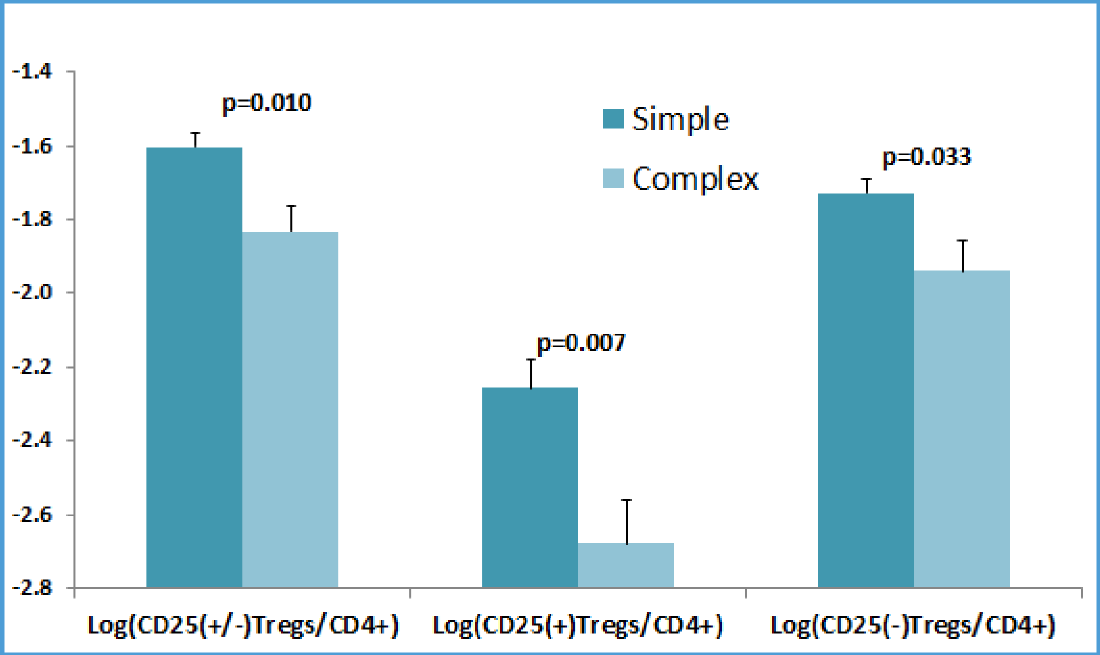
Gene expression analysis – ileal mucosa

Table GO --> CA enriched compared to SA (FDR < 5%)		
Top10	**Name**	**General Proces**
1	ALCOHOL METABOLIC PROCESS	Metabolic
2	T CELL ACTIVATION	Immune
3	GTPASE ACTIVITY	Metabolic
4	CYTOKINE METABOLIC PROCESS	Immune/Metabolic
5	G PROTEIN SIGNALING	Metabolic
6	CYTOKINE ACTIVITY	Immune
7	LYMPHOCYTE ACTIVATION	Immune
8	CYTOKINE BIOSYNTHETIC PROCESS	Immune/Metabolic
9	REGULATION OF LYMPHOCYTE ACTIVATION	Immune
10	GUANYL NUCLEOTIDE BINDING	Metabolic

T-reg cells in the distal jejunal mucosa





Christoforidou et al. 2013

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Effects of a diet intervention

To compare the effects of a simple versus complex starter microbiota and to determine the effects of early pre-weaning nutrition (supply of a diet with or without medium chain triglycerides) on piglet development, gut immunity, microbial development in the gastro-intestinal tract and gut functionality using of CD-derived piglets

Medium chain triglycerides (MCT)

- Coconut oil and palmkernel oil
- High in C6-C12 fatty acids
- Fast intraluminal hydrolysis in gut
- Absorption via blood (fast source of energy)
- Effects on immune system (reduction cytokine response (IL-8), modifies response to LPS, increase IgA secretion)
- Influence intestinal microbiota (antimicrobial properties)

Fatty acid composition coconut oil

Fatty acid	C and =	% in oil
Capric Acid	10:0	6-7
Lauric acid	12:0	49
Myristic Acid	14:0	17,5
Palmitic Acid	16:0	9,0
Stearic Acid	18:0	3,0
Oleic Acid	18:1 (ω-9)	5,0
Linoleic Acid	18:2 (ω-6)	1,8

Treatments

The piglets received colostrum (100 ml) on the day of birth.

At day 3 and 4 each piglet were subjected to one of the two starter flora treatments.

- Treatment SA (n=28, 4 pens with 7 piglets each), associated with a simple microflora on day 1, 2 and 3 and kept in a clean room under SPF conditions.
- Treatment CA (n=28, 4 pens with 7 piglets each), associated with a simple microflora on day 1, 2 and 3 and subsequently with a complex microflora on day 3 and 4 and kept in a clean room under SPF conditions.

  These piglets receive a fixed amount of diluted faeces from a conventional sow from a different farm as the farm used for the sows providing the CD piglets[1].

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Experimental treatments

1. Treatment SA - SO/PO (n=14, 2 pens with 7 piglets each), associated with a simple microbiota (SA) and fed a moist diet with soya oil (SO) and palm oil (PO)
2. Treatment SA - CO (n=14, 2 pens with 7 piglets each), associated with a simple microbiota (SA) and fed a moist diet with coconut oil (CO)
3. Treatment CA - SO/PO (n=14, 2 pens with 7 piglets each), associated with a simple microbiota and subsequently with a complex microbiota (CA) and fed a moist diet with soya oil (SO) and palm oil (PO)
4. Treatment CA - CO (n=14, 2 pens with 7 piglets each), associated with a simple microbiota and subsequently with a complex microbiota (CA) and fed a moist diet with coconut oil (CO)

Diet composition (g/kg)

	Control	MCT
Maize	200.0	200.0
Whey Powder	180.0	180.0
Wheat	150.0	150.0
Oats Flocs	80.0	80.0
Barley	70.0	70.0
Wheat gluten meal	50.0	50.0
Full fat soybeans	40.0	40.0
Soycomill-P	33.0	33.0
Lactose	30.0	30.0
Potato protein	30.0	30.0
Monocalciumphosphate	0.6	0.6
Rice Meal	12.0	12.0
Limestone	0.9	0.9
Salt	3.6	3.6
Dicalciumphosphate	19.4	19.4
Premix	5.0	5.0
Soya oil	50.6	3.8
Palm oil	30.0	6.8
Coconut oil		70.0

	Control	MCT
DM	914	914
Ash	54	54
CP	195	195
EE	115	115
Crude fibre	20	20
Starch	245	245
Sugars	141	141
Ca	7.0	7.0
Dig. P	4.8	4.8
K	3.5	3.5
Na	3.5	3.5
Cl	3.9	3.9
dEB (meq/kg)	175	175
NEpigs (MJ/kg)	12.20	12.20

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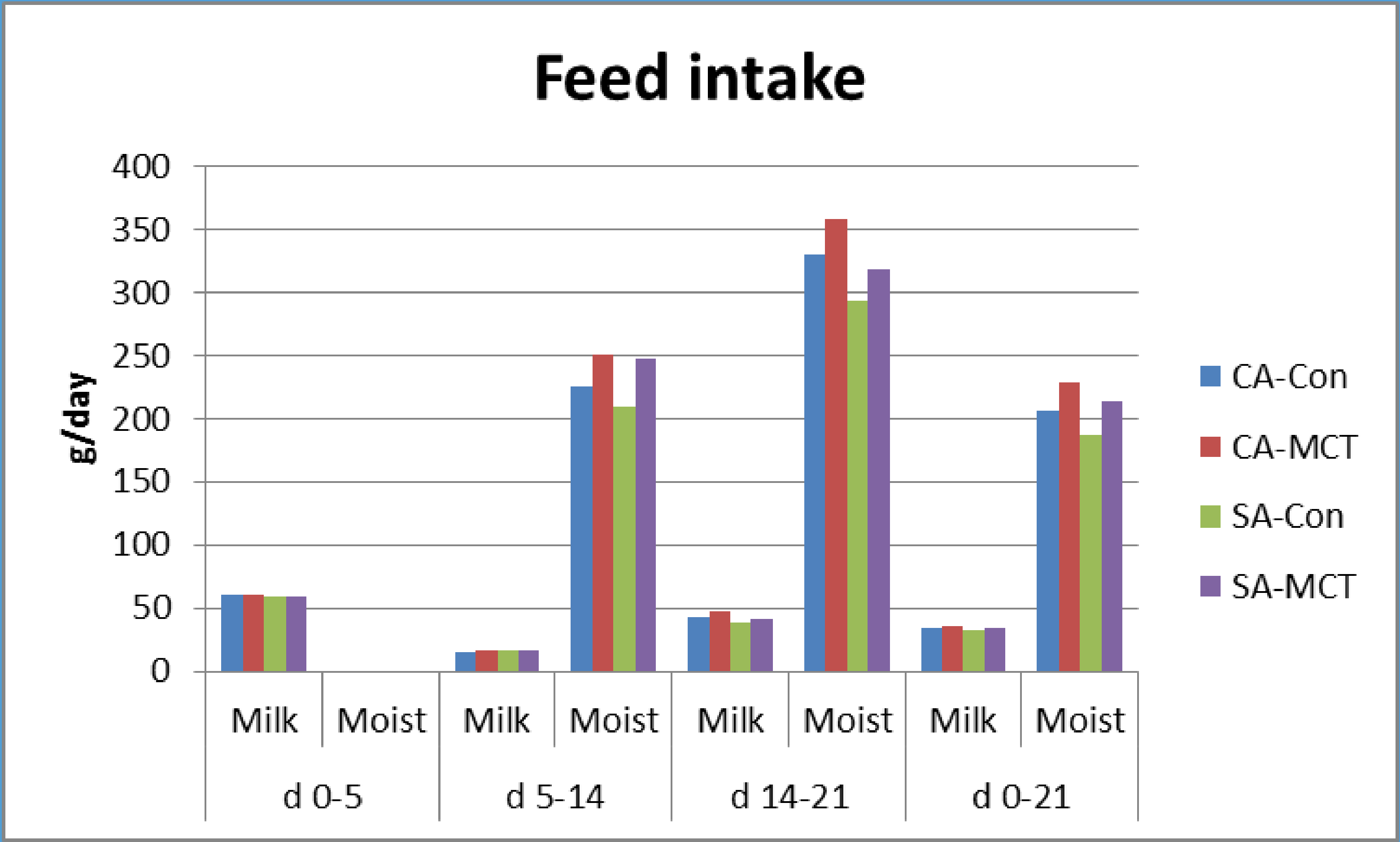
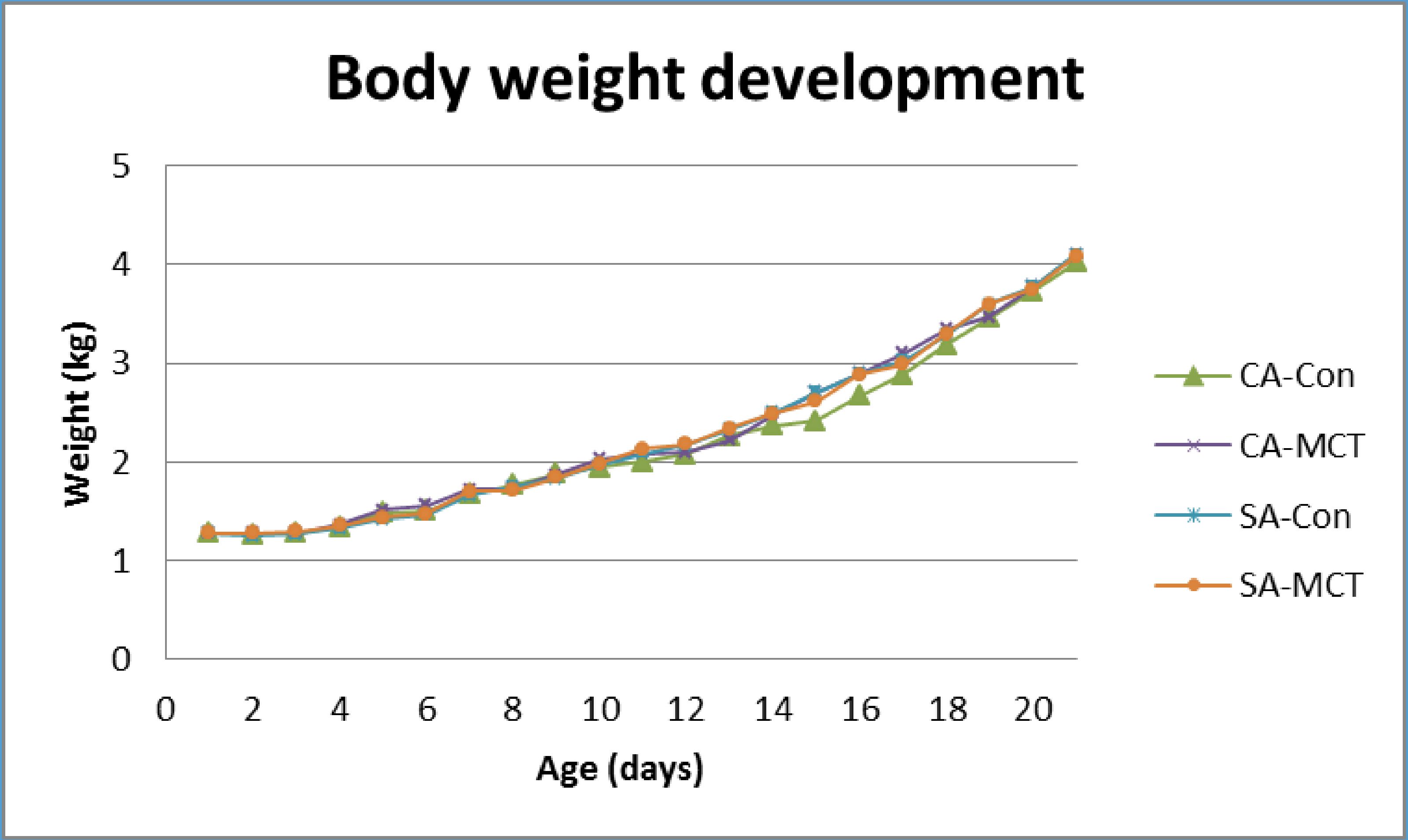
Automix

Effects of treatments of feed intake

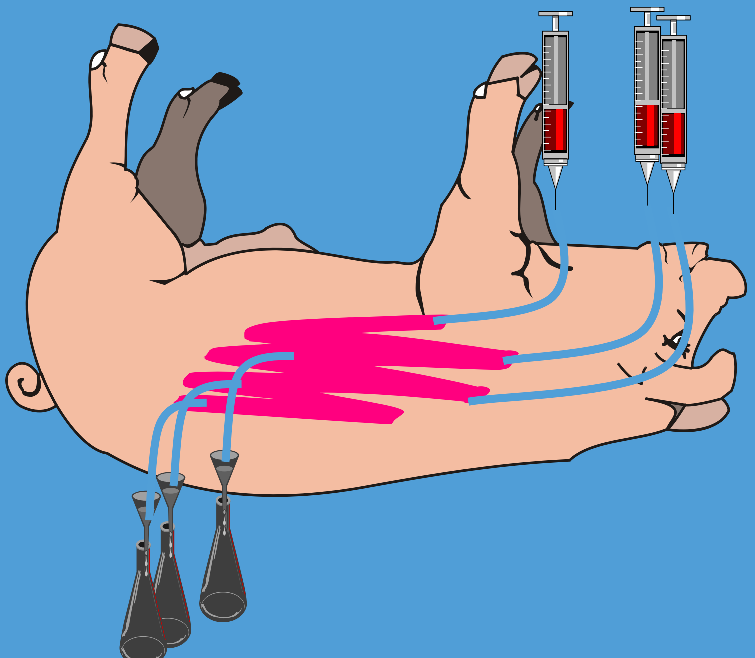


Development of body weight



In situ: Small Intestinal Segment Perfusion (SISP) system

- Pigs under anaesthesia
- 10 segments in small intestine (± 20 cm long)
- cranial inflow tube (∅ 3 mm), caudal outflow tube (∅ 5 mm)
- Perfusion 2 ml per 15 min
- ETEC challenge 5 ml, 1.1 x 10^9 cfu/ml



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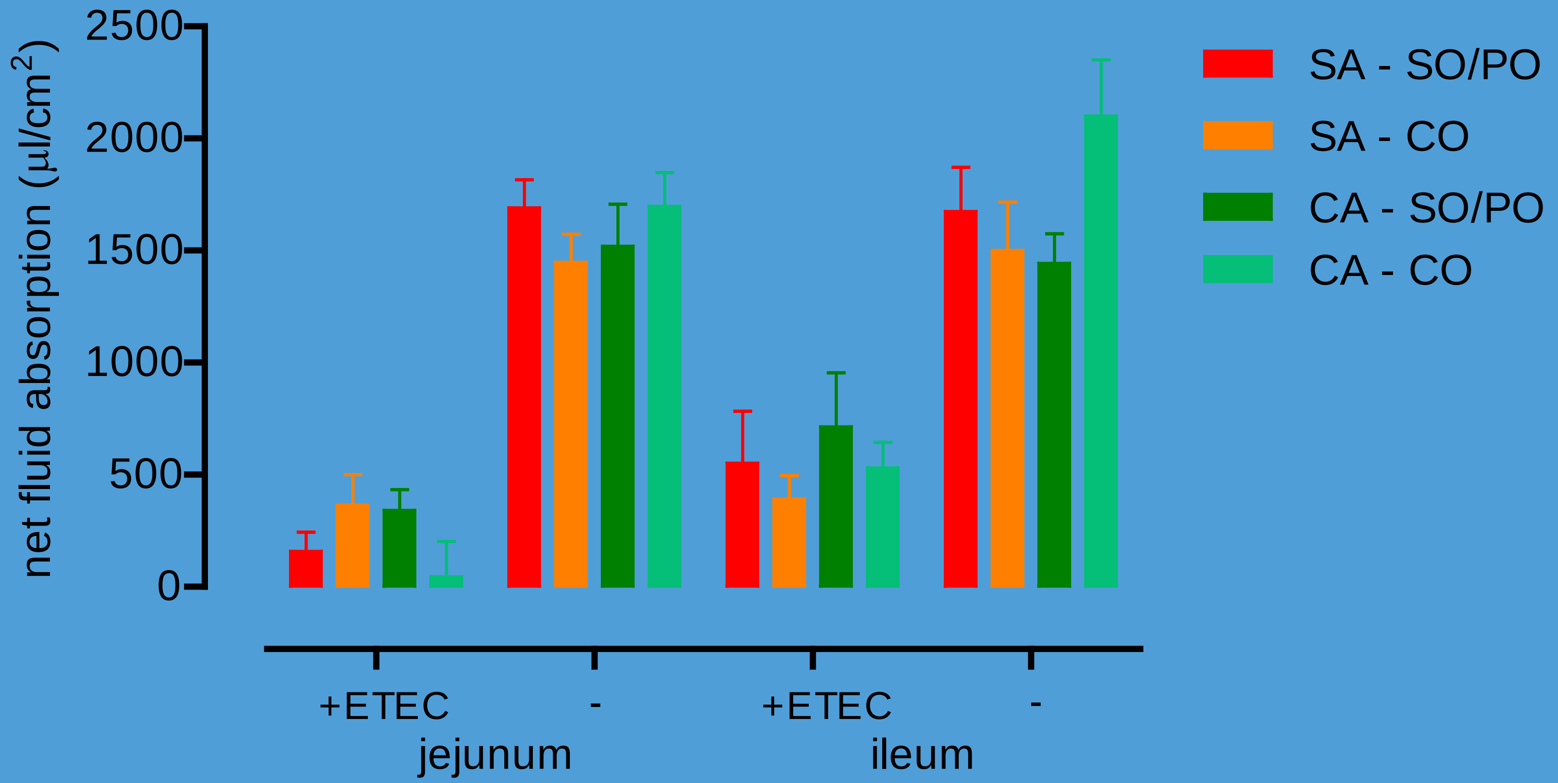
Treatments evaluated

Treatment	Conditions	n	Challenge	Location
Saline	-	24	-/+ ETEC	Jejunum
Saline	-	24	-/+ ETEC	Ileum
Coconut oil	+ lipase	24	-/+ ETEC	Jejunum
C10/C12	+ lipase	12	-/+ ETEC	Jejunum
GRL1110 (L. amylovorus)	5 ml Lacto, 1.4 x 10^9 cfu/ml, saline after 1 h ETEC	6	-/+ ETEC	Jejunum
GRL1112 (Lactobacillus)	5 ml Lacto, 1.7 x 10^9 cfu/ml, saline after 1 h ETEC	6	-/+ ETEC	Jejunum
GRL1110 (L. amylovorus)	5 ml 1.6 x 10^8 per ml, saline	12	- ETEC	Jejunum
E. coli fimbriae	5 ml 0.05 mg/ml, perfused 0.004 mg/ml in saline	12	- ETEC	Jejunum

Fluid absorption as affected by treatment



Saline perfusion (n=24)

Association: NS; Diet : NS

ETEC: P<0.001

Site: P<0.05 (absorption is higher in ileum than in jejunum)

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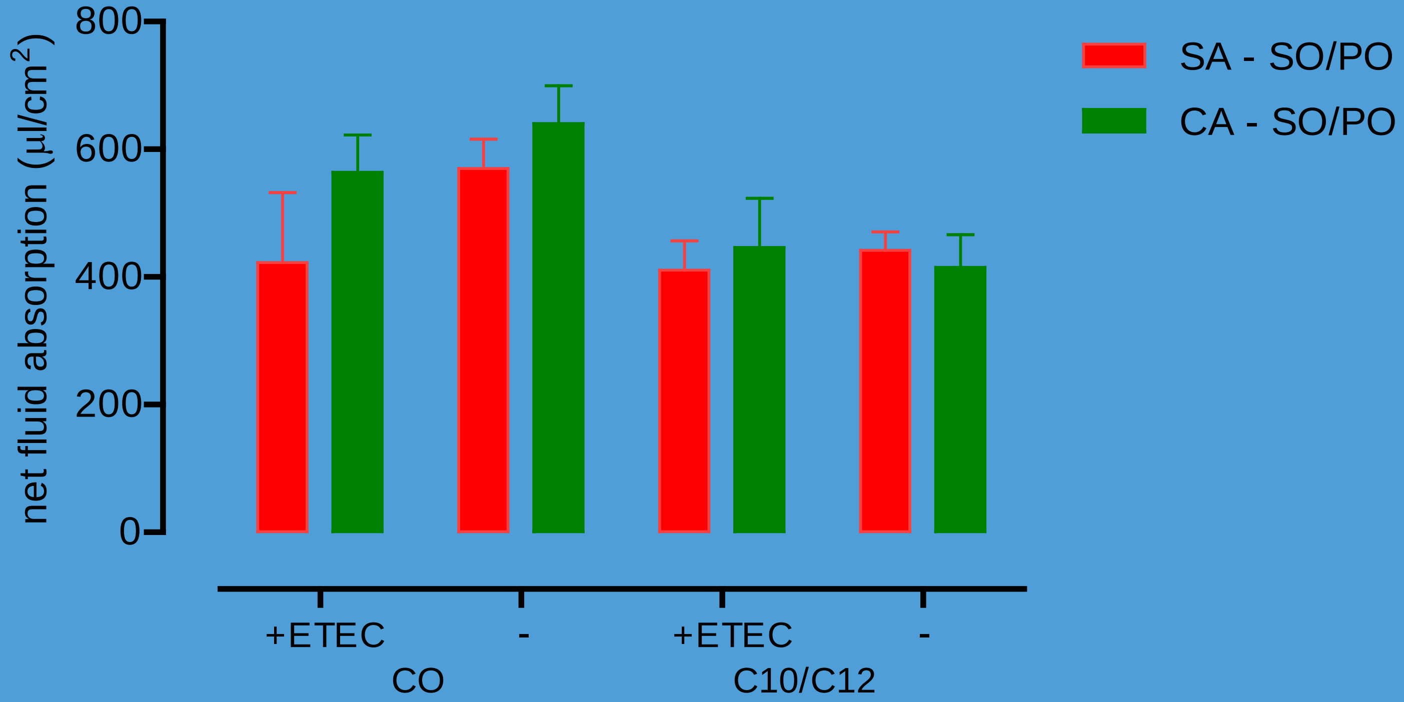
Fluid absorption as affected by treatment



n=12

Association: ns

Perfusion: P=0.002 (C10/C12 lower than CO)

ETEC: ns

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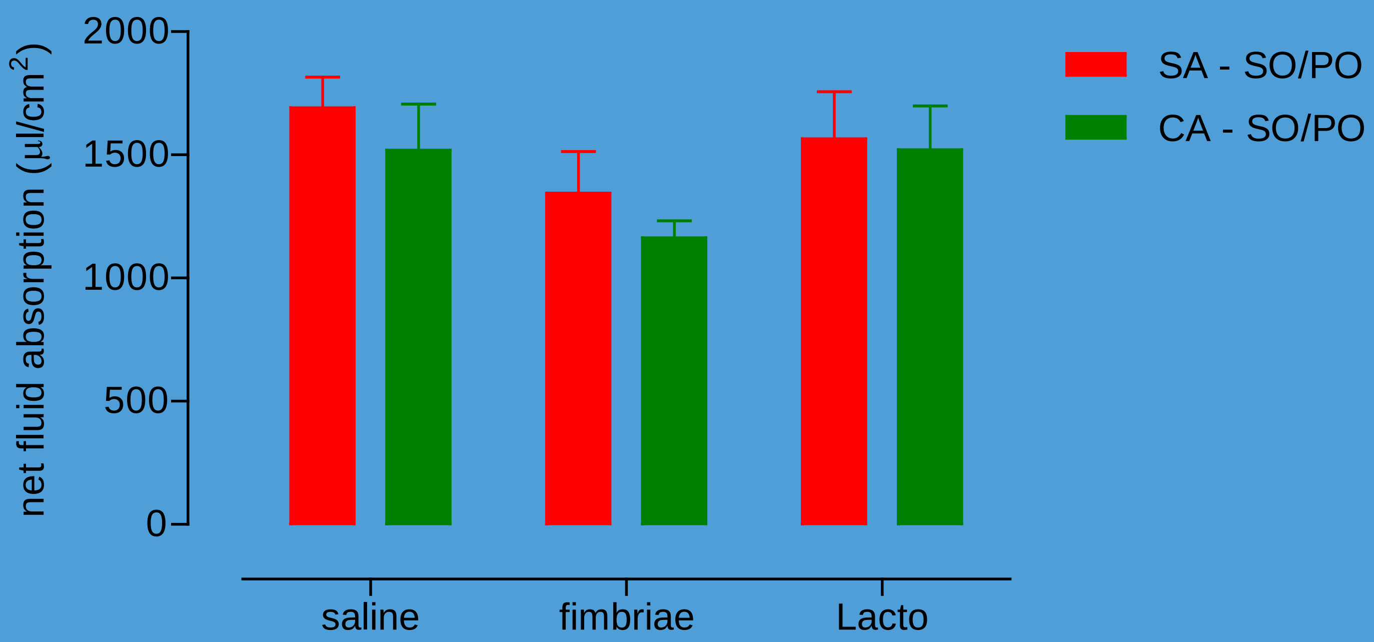
Fluid absorption as affected by treatment

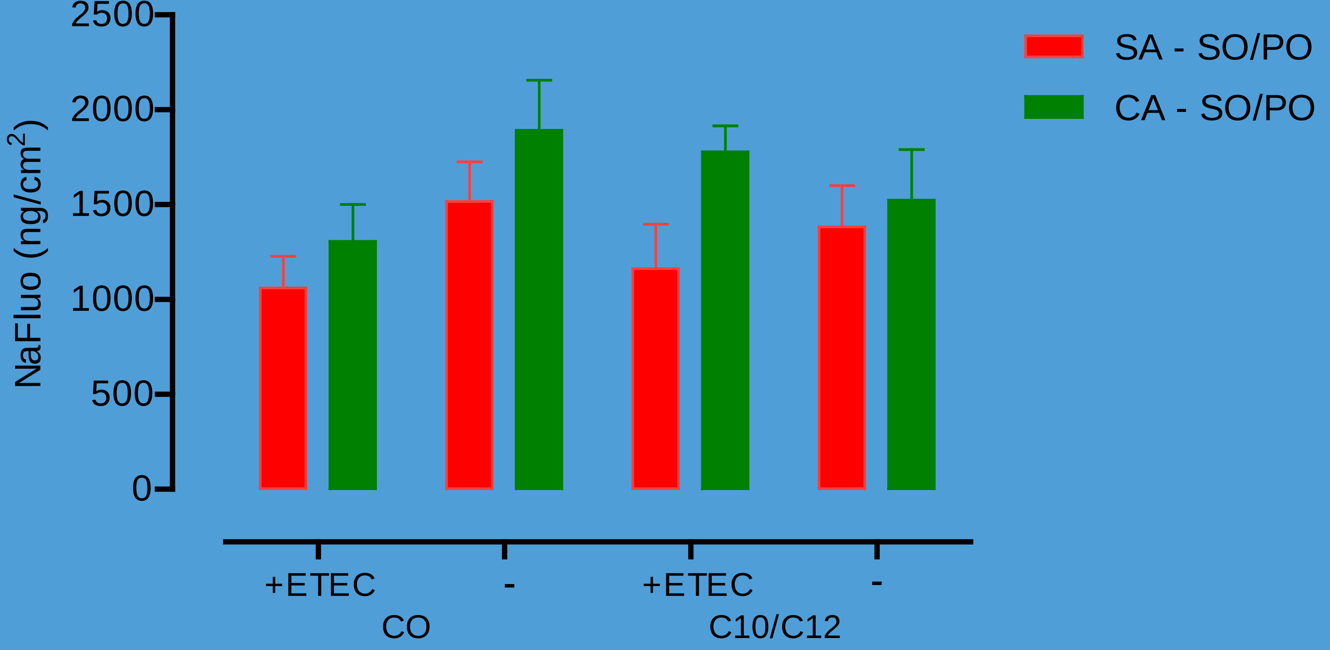


n=12

Association: ns

Treatment: P=0.010 (fimbria < Lacto & Saline)

Permeability as affected by treatment



n=12

P association: 0.086

Perfusion: ns

P ETEC: 0.084

Lallès al. 2013

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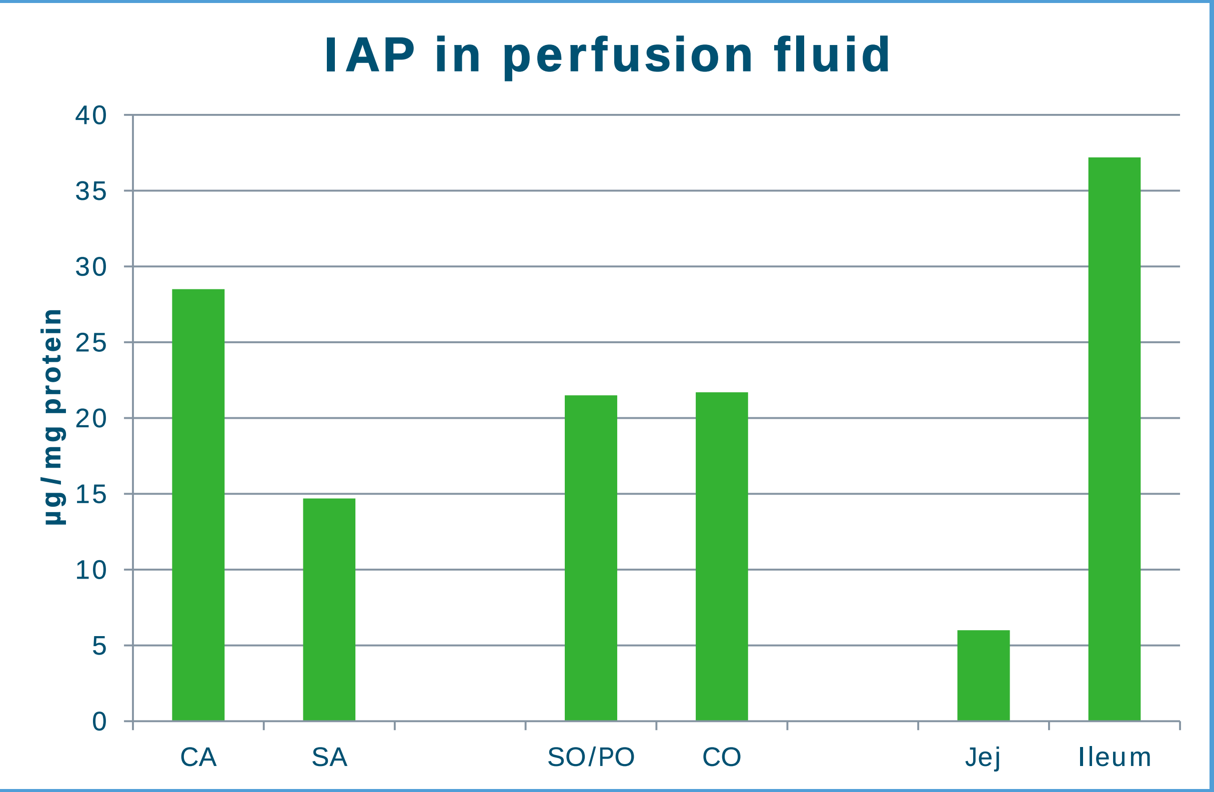
Intestinal alkaline phosphatase



Association: P = 0.02

Diet: NS

Site: P<0 001

Association x Site: P = 0.04

Lallès al. 2013

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Conclusions

- Microbiota composition in the digestive tract of piglets in later life is influenced in complexity and composition by the early life environment.

- The microbiota composition in the GI tract of pigs has functional consequences on the various functions of the gut (nutrient absorption, barrier function, important component of the immune system) and can have long term effects on health and performance of the pig throughout life.

- More knowledge needs to be acquired on appropriate ways to actively intervene with the gut microbiota composition at young age.

Acknowledgements

EU - INTERPLAY

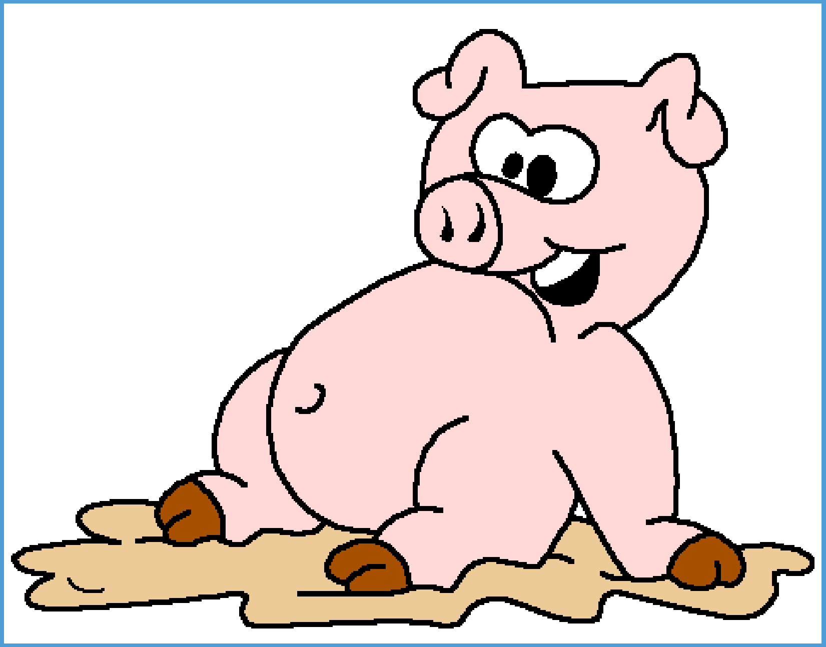
Interplay of microbiota and gut function in the developing pig – Innovative avenues towards sustainable animal production

Dutch Commodity Board Animal Feed (PDV)

PRODUCTSCHAP DIERVOEDER

University of Bristol (UK)

Thank you for your attention!



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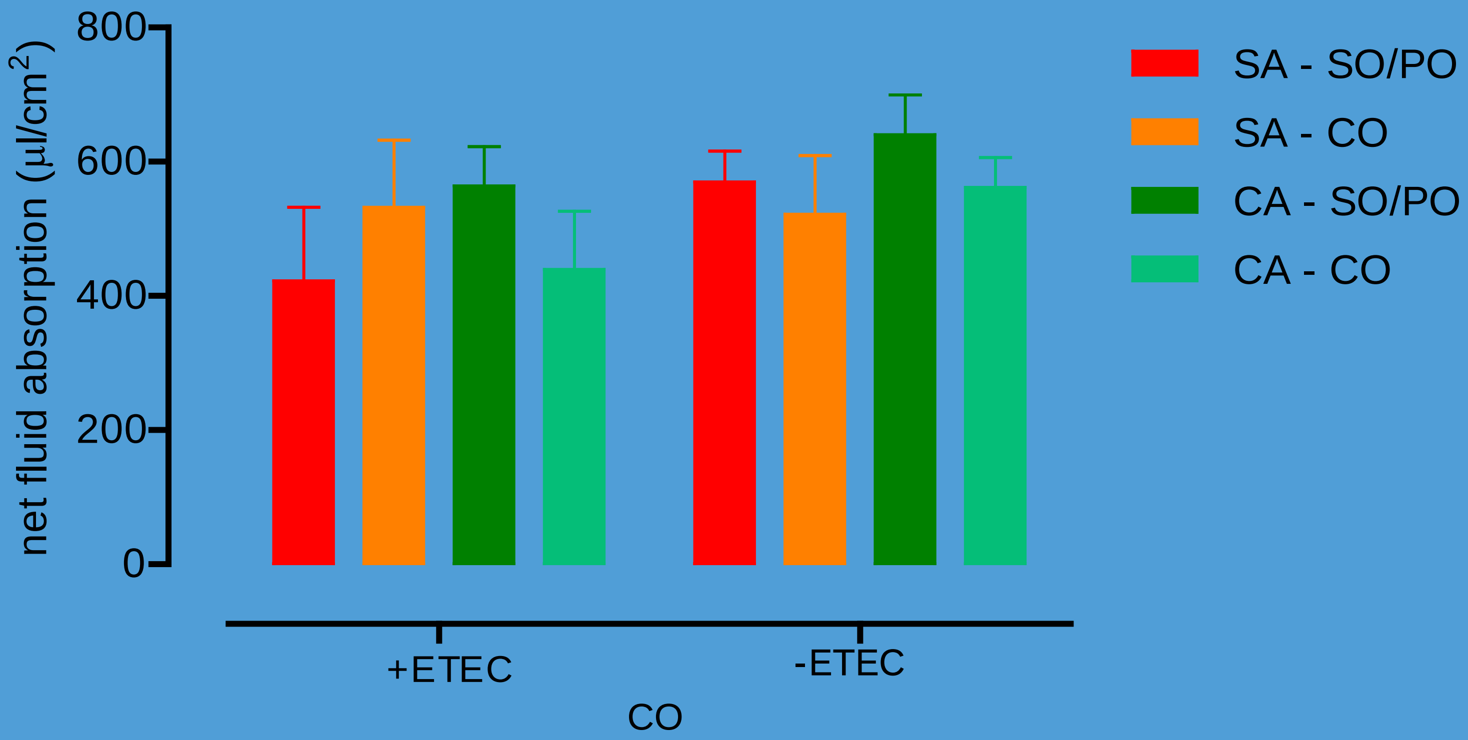
Fluid absorption as affected by treatment



Coconut oil perfusion (n=24)

Association: NS; Diet : NS

ETEC: 0.051 (CO reduces the effect of ETEC; CO absorption is low)

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