The role of the intestinal microbiota in gut health in pre- and post-weaning piglets

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Functions of the gut

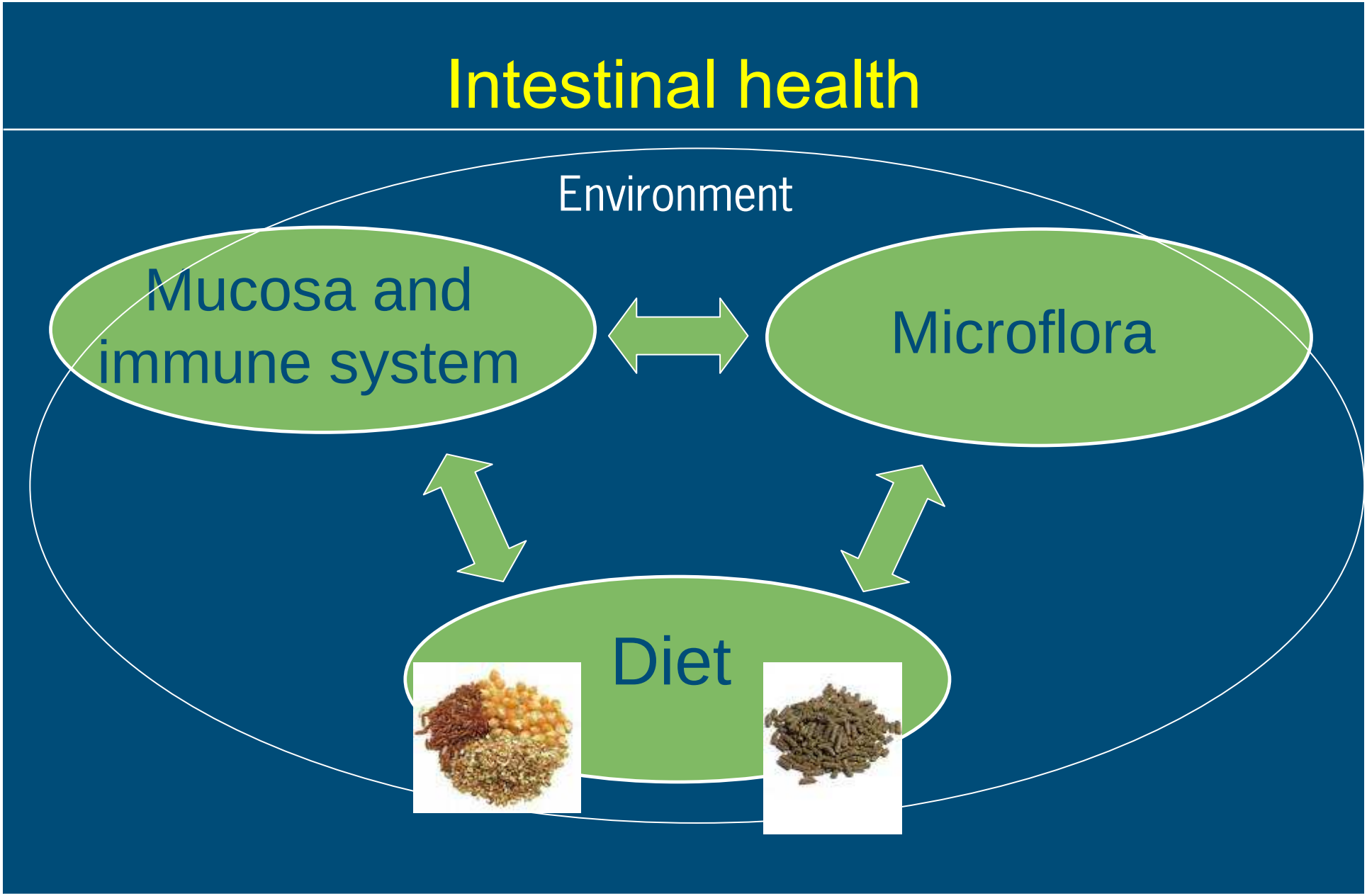
Nutrient digestion

- Enzymatic digestion
- Fermentative digestion

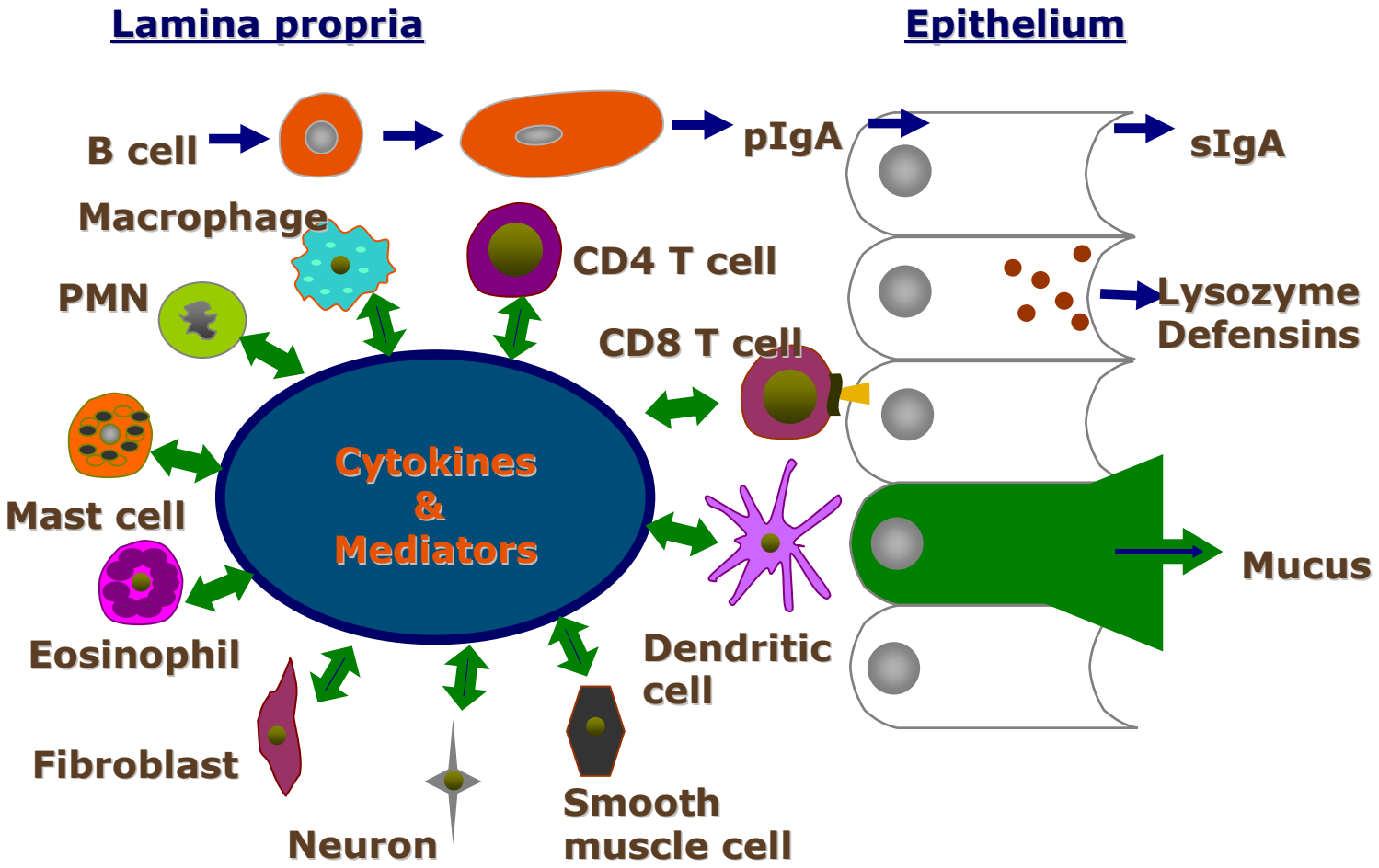
Barrier function

- Intestinal microflora
- Immune system

Intestinal health

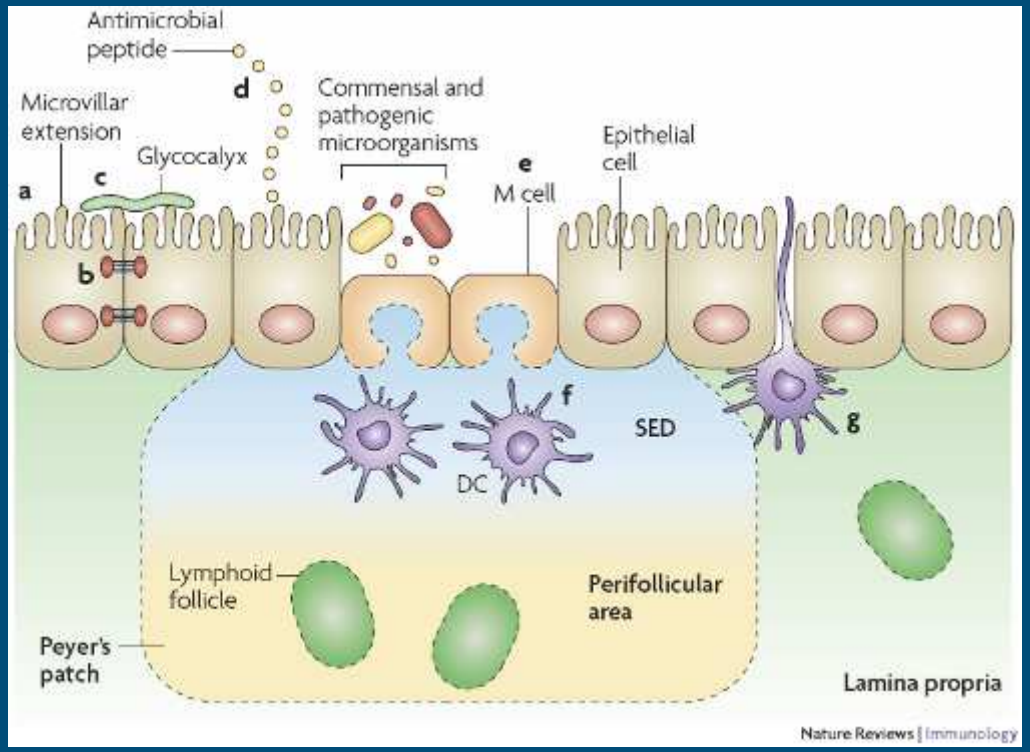


Cells in the gut mucosa



Gaskins, 2009

Gut barrier function



Simple columnar epithelial cells exhibit physical and biochemical adaptations to maintain barrier integrity including actin-rich microvillar extensions (**a**), epithelial-cell tight junctions (**b**), apically attached and secreted mucins that form a **glycocalyx** (**c**) and the production of various antimicrobial peptides (**d**). Specialized intestinal epithelial cells known as M (microfold) cells overlie Peyer's patches and lymphoid follicles to facilitate luminal sampling. M cells exhibit reduced mucin secretion and have modified apical and basolateral surfaces (**e**) to promote uptake and transport of luminal contents to professional antigen-presenting cells that inhabit the subepithelial dome (SED) of the Peyer's patches and lymphoid follicles (**f**). Specialized dendritic cell (DC) subsets can also extend dendrites between the tight junctions of intestinal epithelial cells to sample luminal contents (**g**).

Arts, 2008

Introduction

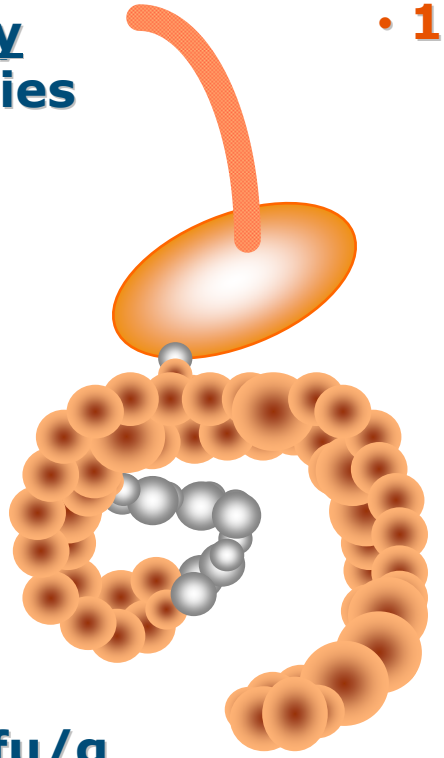
- Gut function
- Microbiota in the gut of pigs
- Microbial succession in the gut
- Microbiota and gut development
- Microbiota and the immune system
- Effects of weaning
- Effects of diet composition (ingredients and additives)
- EU FP 7 Interplay – setup and some preliminary results
- Conclusions

Microflora in the gut

- 10^{13} cells in the body
- 10^{14} GI bacteria

Oral Cavity
- 200 species

Stomach
- *H. pylori*

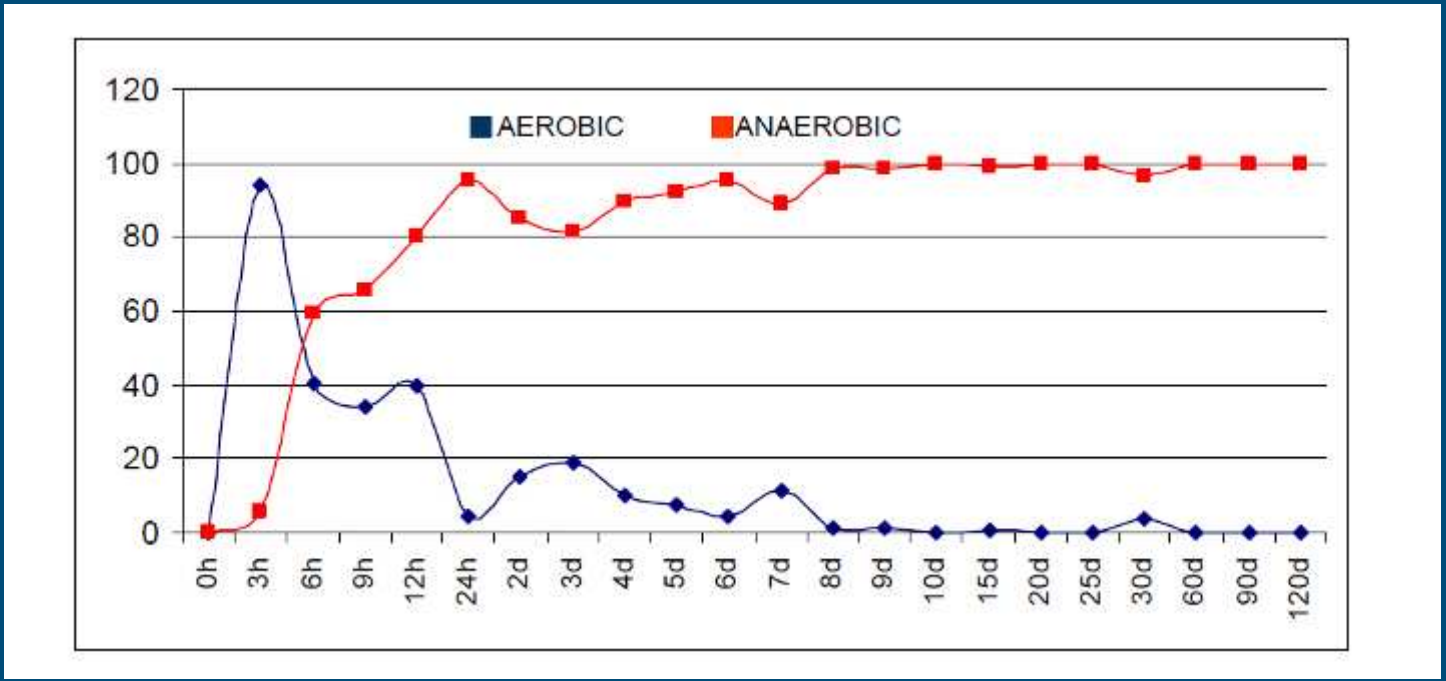


Ileum
- 10^{8} cfu/g

Large Intestine
- 10^{11} - 10^{12} cfu/g
- 300 - 400 species
- *Bacteroidetes*
- *Firmicutes*
- *Proteobacteria*
- *Actinobacteria*
- *Cyanobacteria*
- *Fusobacteria*
- *VadinBE97*
- *Verrucomicrobia*

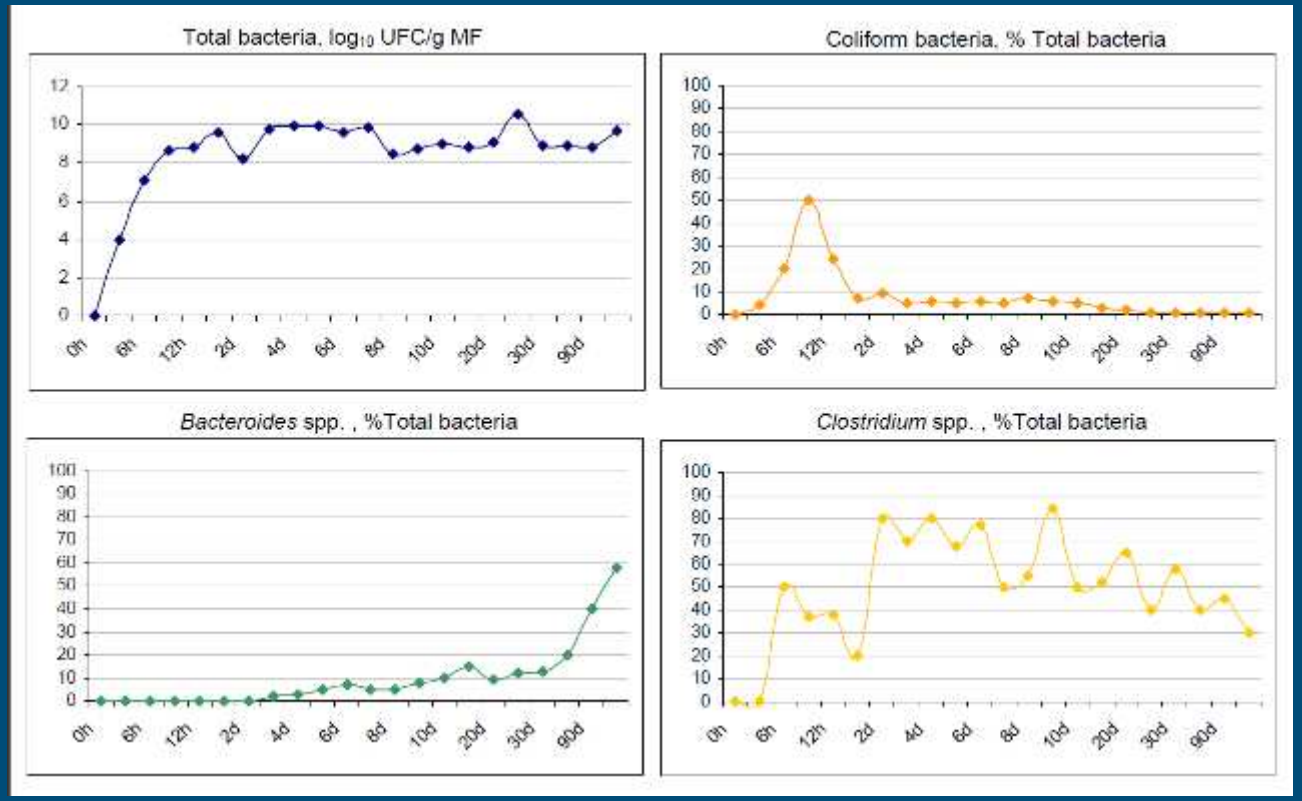
Gaskins, 2009

Evolution of aerobic and anaerobic bacteria in the faeces from birth to 120 d of life (% of total)



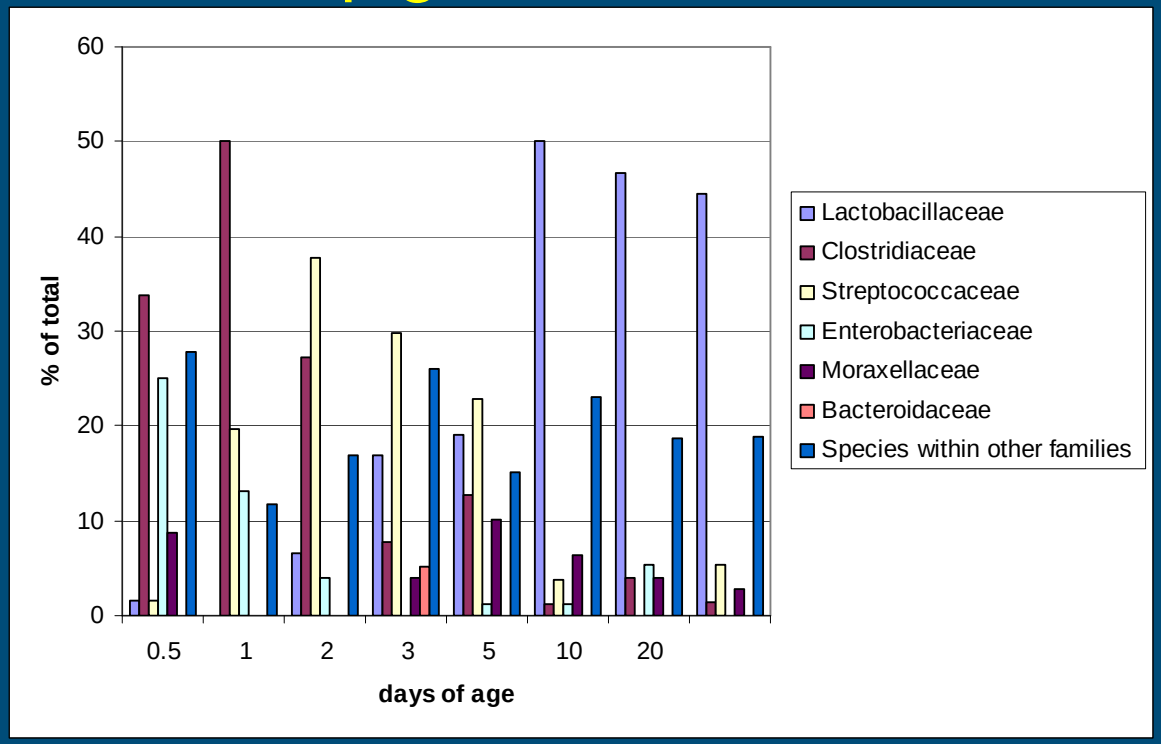
Castillo Gomez, 2006

Evolution of faecal bacteria in piglets from birth till 120 d of life (cfu/g and % of total)



Castillo Gomez, 2006

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

Environment and microbial colonisation of the gut

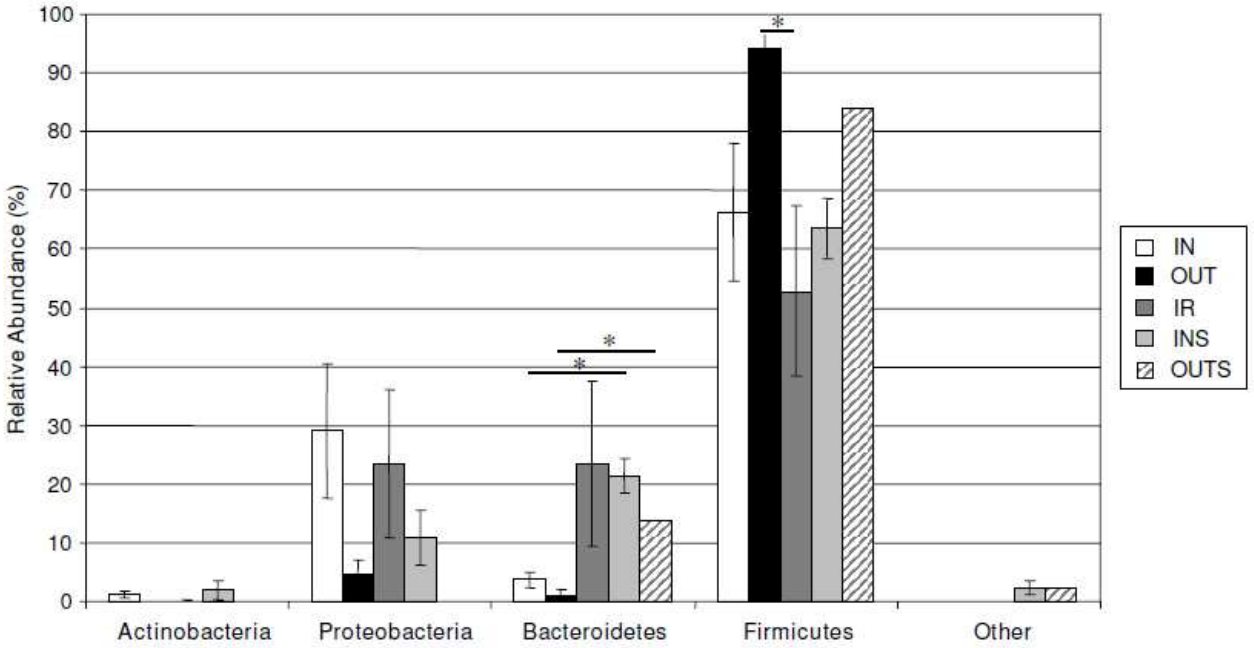


Figure 3
Phylogenetic distribution of clones obtained from mucosa-adherent ileal and fecal samples in different housing environments. The majority of clones were assigned to the phyla Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria. The Firmicutes phylum was significantly increased in the OUT group compared to the IR group ($P < 0.05$). The Bacteroides phylum was significantly increased in both the INS and OUTS fecal libraries compared to the mucosa-adherent ileal libraries. Values are expressed as means ± SEM (N = 4).

Mulder et al., 2009

Environment and microbial colonisation of the gut

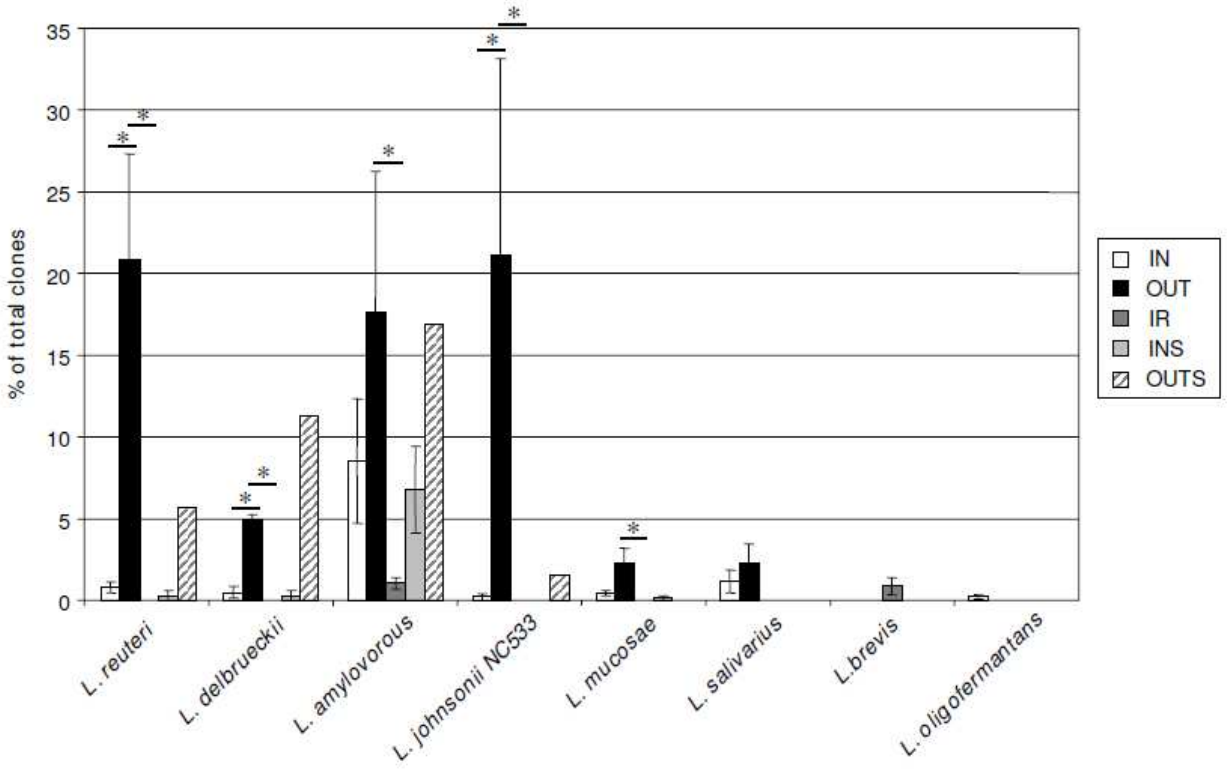
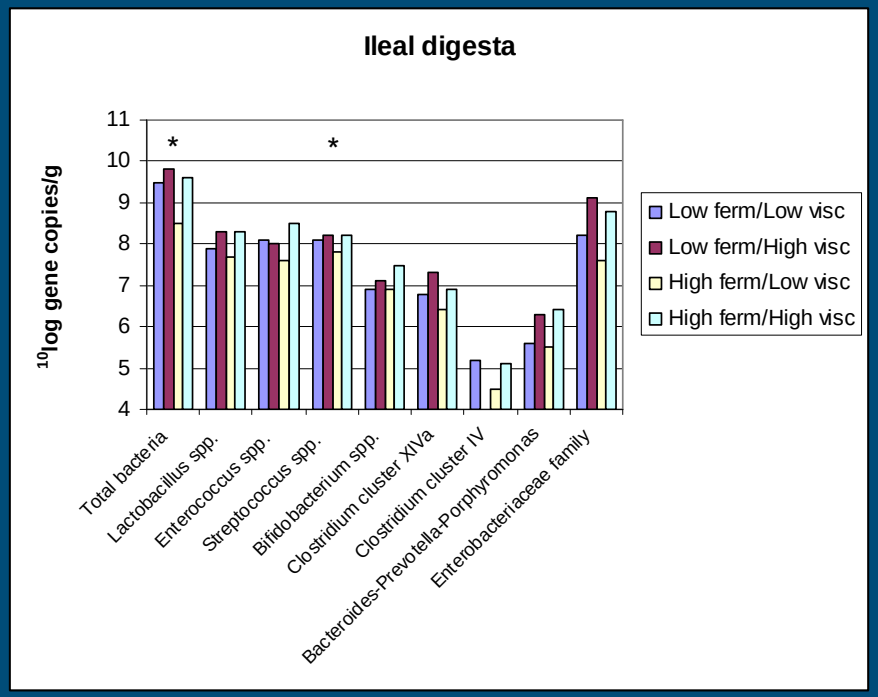
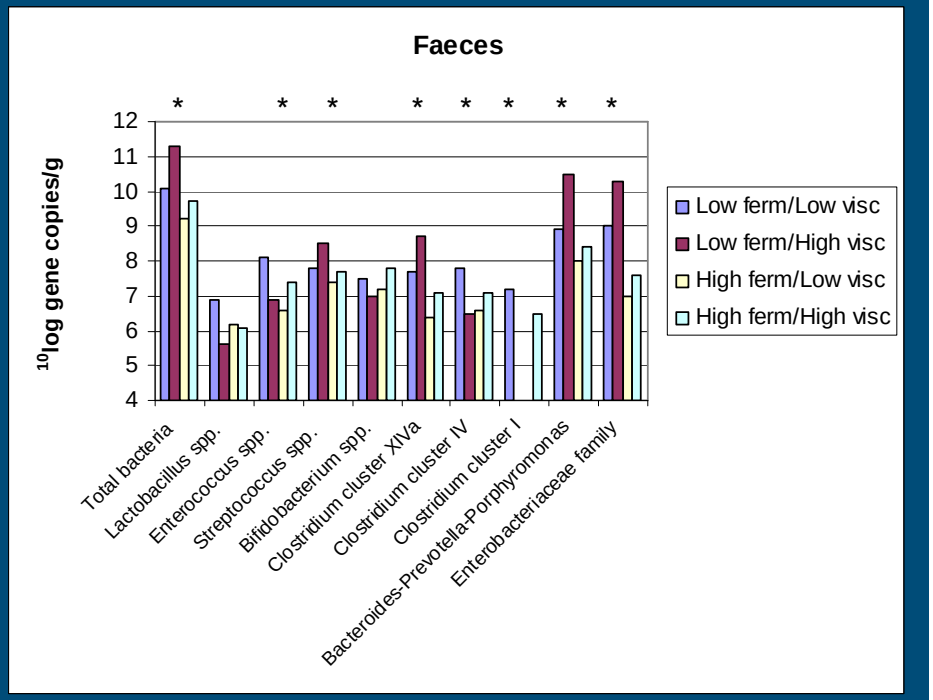


Figure 4
Abundance of lactobacilli in mucosa-adherent ileal and fecal samples in different housing environments. The Lactobacillaceae family included *L. reuteri*, *L. amylovorous* LAB31, *L. johnsonii*, *L. delbrueckii* subsp. *bulgaricus*, *L salivarius* and *L. mucosae*. *L. reuteri*, *L. delbrueckii* and *L. johnsonii* were all significantly lower in the IN and IR groups compared to the OUT group. Values are expressed as means ± SEM (N = 4).

Mulder et al., 2009

Bacterial groups in ileal digesta and faeces as affected by viscous and fermentable NSP in growing pigs





By dietary inclusion of cellulose, carboxymethylcellulose (CMC), high-fermentable, low-viscosity oat ß-glucans and high-fermentable, high-viscosity oat ß-glucans.

Metzler-Zebeli et al., 2010

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Host microbiota interplay

- Gut bacteria drive and maintain immune development and function
- Millions of years of host/microbe evolution and adaptation
- Mutualism and symbiosis
- A healthy gut relies on appropriate interactions with bacteria
- Interaction between host gut and gut bacteria must be regulated
- Inappropriate regulation and immune responses are triggers for gut dysfunction
- Microbial balance is critical

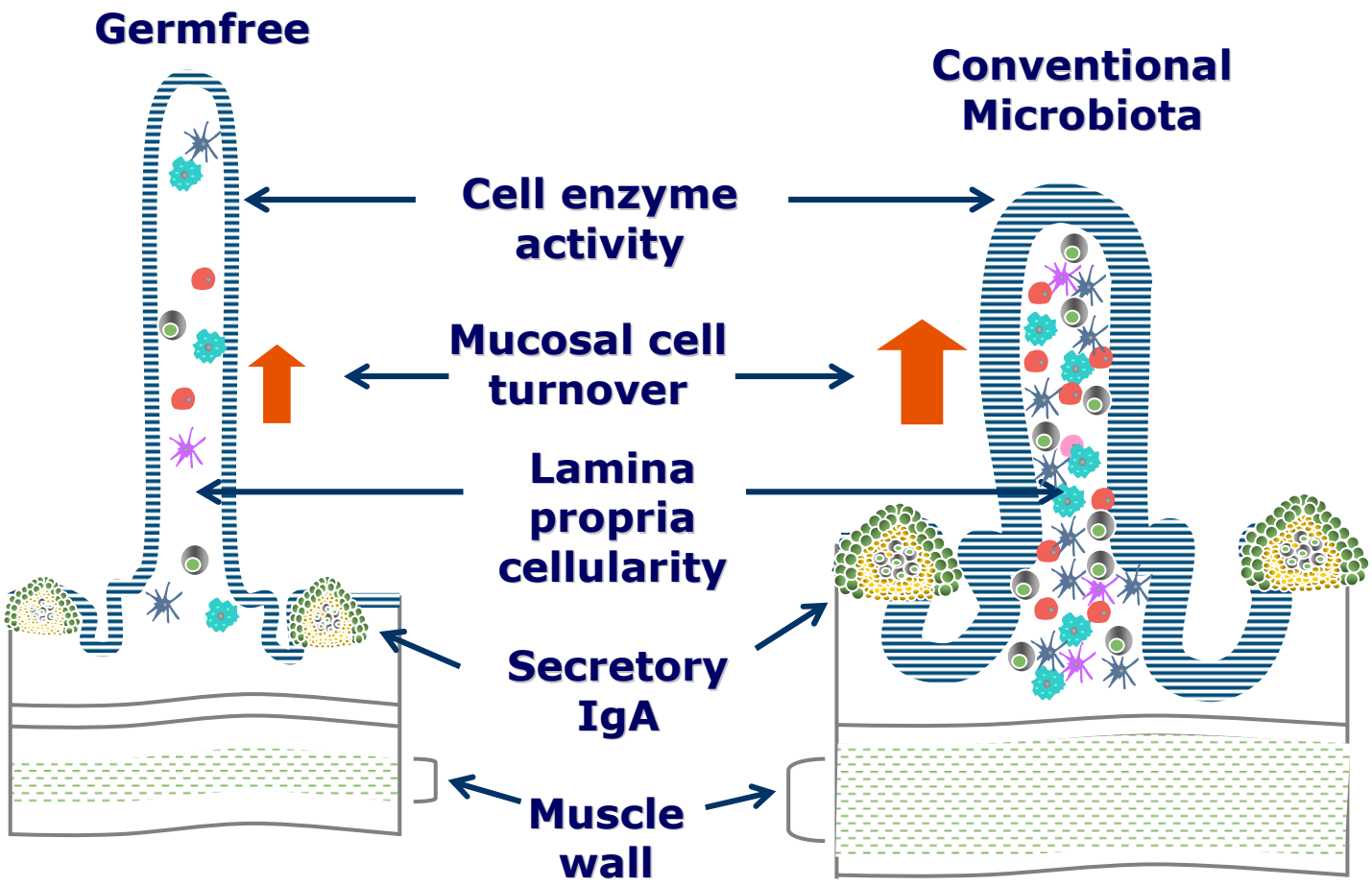
Kelly, 2009

Functions of the intestinal microflora

Protective functions	Structural functions	Metabolic functions
Pathogen displacement	Barrier fortifications	Ferment non-digestible dietary residue and endogenous epithelial-derived mucus
Nutrient competition	Induction of IgA	Synthesize vitamins, e.g., biotin, folate, vitamin K
Receptor competition	Apical tightening of tight junctions	Control intestinal epithelial cell differentiation and proliferation
Production of antimicrobial factors, e.g., bacteriocins, lactic acids	Immune system development	Ion absorption

Salzman et al., 2007

Gut mucosa morphology



Gaskins, 2009

Host microbial response pathways

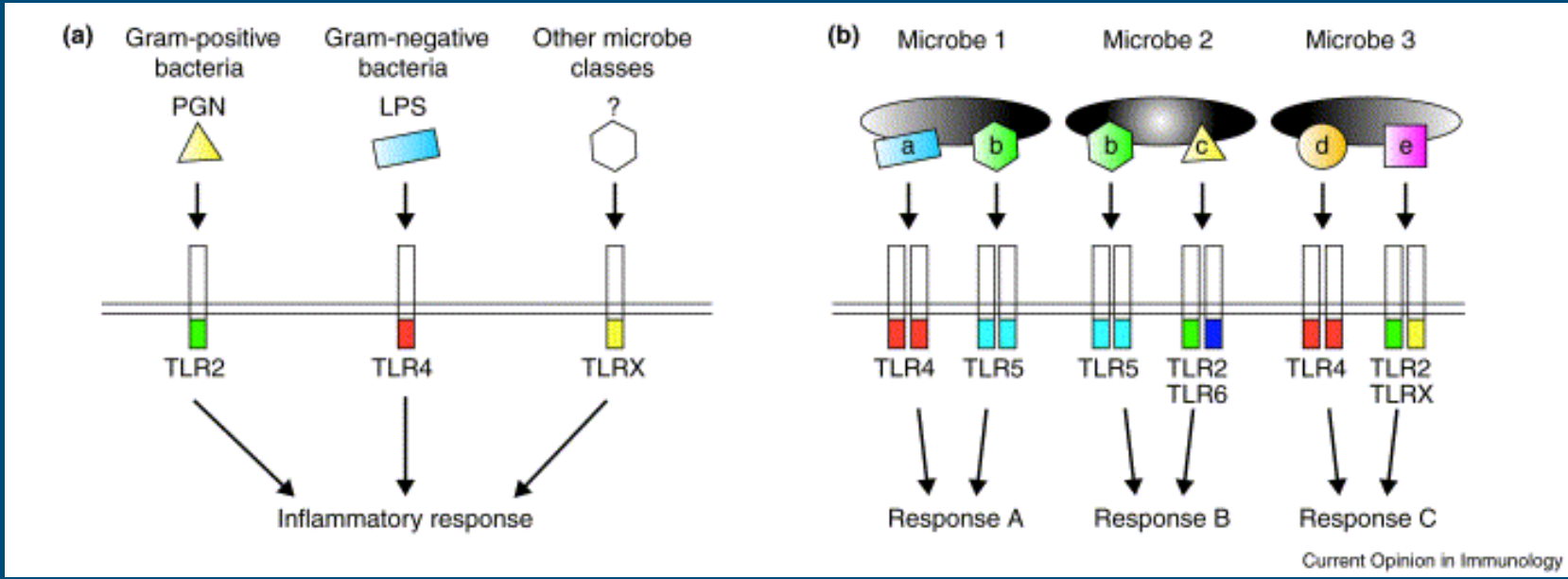
- Microbial antigen sampling and activation of specific immunity in Peyer's Patches
- Receptor mediated recognition of microbiota-associated molecular patterns (TLRs, Nod family receptors, galectine familiy receptors)
- Host metabolism of microbial fermentation products
- Host receptor recognition of microbial-origin fermentation products (SCFA, lactic acid, digestion products of protein)

Van Kessel, 2009

Toll like receptors and microbe recognition

Early model

Revised model

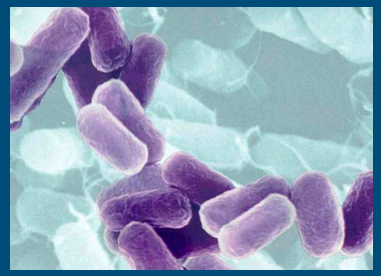


Underhill and Ozinsky, 2002

Host-microbe interactions

Microbe-associated Molecular Patterns (MAMPs)

- **Lipopolysaccharides**
- **Peptidoglycans**
- **Lipoproteins**

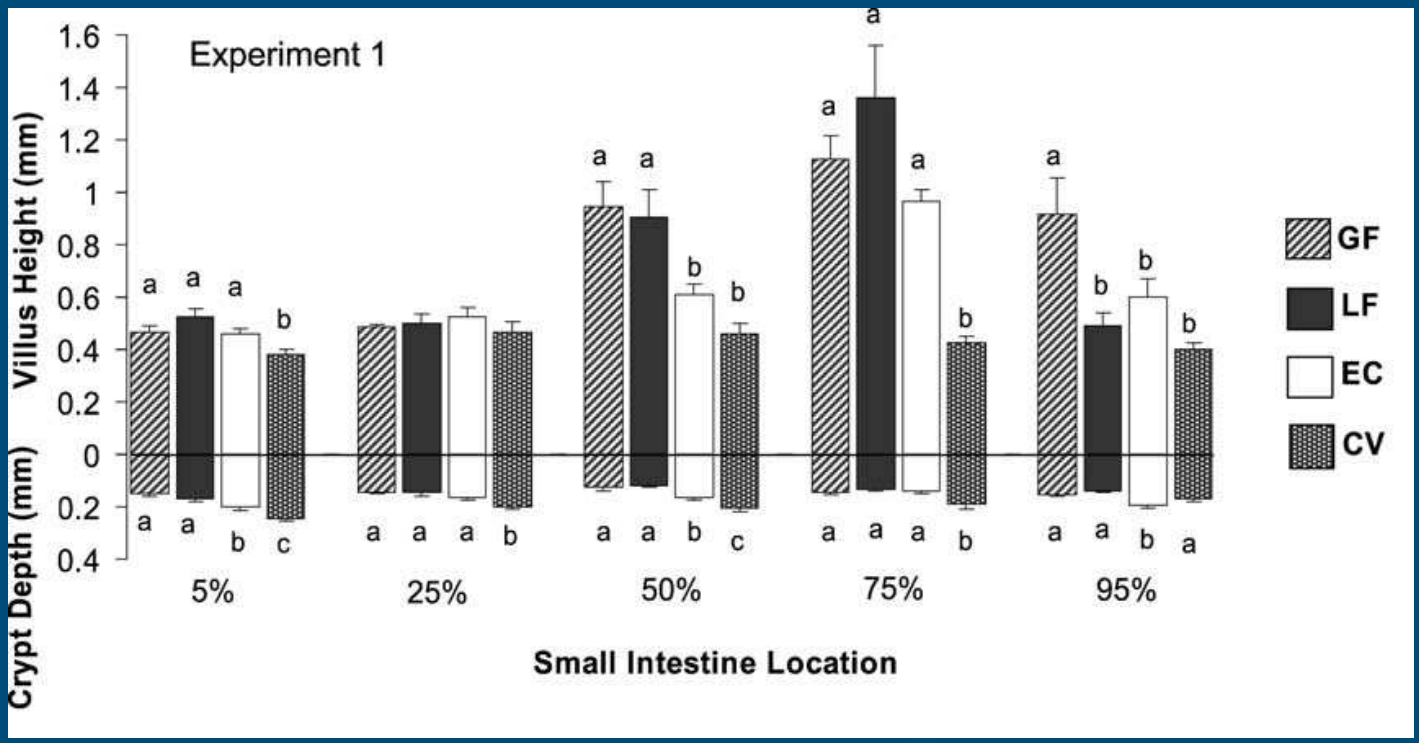


Pattern Recognition Receptors (PRRs)

- **C-type lectins**
- **Leucine rich proteins (Toll like receptors)**
- **Lipid transferases**
- **Integrins**
- **Scavenger receptors**
- **Pentraxins**

Microflora and gut development

Villus morphology as affected by gut microbial colonization



Mean villus height (upper bars) and crypt depth (lower bars) measured from 10 well-oriented villi and crypts from 5% to 95% of the length of the SI (pyloric sphincter 0%; ileo-cecal junction100%) in pigs derived by cesarian section. In the experiment pigs were germ-free (GF), or inoculated with either L. fermentum (LF), a nonpathogenic E. coli (EC), or conventionalized with fresh adult porcine fecal material (CV).

Shirkey et al., 2006

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Effects of microbiota gene expression in gut tissue

Table 3. Mean fold change in gene expression relative to germ-free (GF) in whole tissue at 75% of small intestinal length in 14-d-old gnotobiotic pigs (Exp. 1 and 2)

	Gene of interest[1]				
Treatment group[2]	FasL	TNFα	TLR2	TLR4	TLR9
Exp. 1					
GF	1.02^{b}	1.03^{b}	1.10^{b}	1.02^{b}	1.04
SF	3.69^{a}	2.69^{a}	4.72^{a}	2.30^{a}	1.77
EC	1.97^{b}	2.12^{a}	2.16^{b}	1.59^{ab}	1.53
LF	0.88^{b}	1.14^{b}	1.00^{b}	1.10^{b}	0.80
Pooled SEM	0.629	0.494	0.475	0.261	0.275
Exp. 2					
GF	1.04^{b}	1.04	1.01^{b}	1.00	1.03
SF	2.73^{a}	1.70	2.46^{a}	1.20	1.10
EC	1.46^{b}	1.38	2.04^{ab}	1.08	0.81
LFKP	2.00^{ab}	1.40	2.47^{a}	0.89	0.88
Pooled SEM	0.326	0.176	0.360	0.190	0.175

$^{a-c}$Means within the same column and experiment with different superscripts are different ($P < 0.05$).
[1]FasL = Fas ligand; TNFα = tumor necrosis factor α; TLR = toll-like receptor.
[2]LF = *Lactobacillus fermentum* and EC = *Escherichia coli* monoassociated pigs; LFKP = LF and *Klebsiella pneumonia* diassociated pigs; SF = conventionalized pigs.

Willing and van Kessel, 2007

Environment and gene expression in the gut

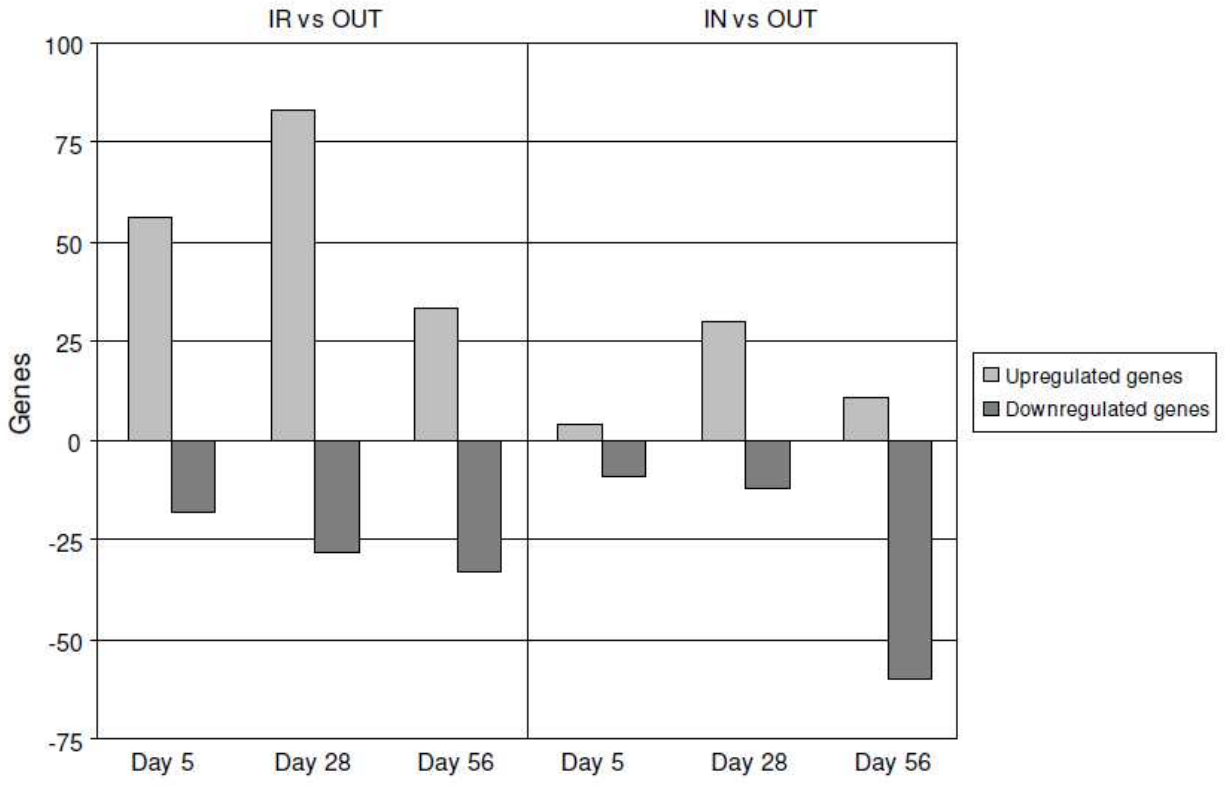

Figure 6
Differentially expressed genes in the ileum of animals housed in different environments. Differentially expressed genes at each time-point are shown for the two treatment comparisons ($P < 0.01$, $-2 \leq$ fold change ≥ 2, $N = 6$). Microbiota differences between the treatment groups were associated with large differences in gene expression in the ileum.

Mulder et al., 2009

Environment and gene expression in the gut

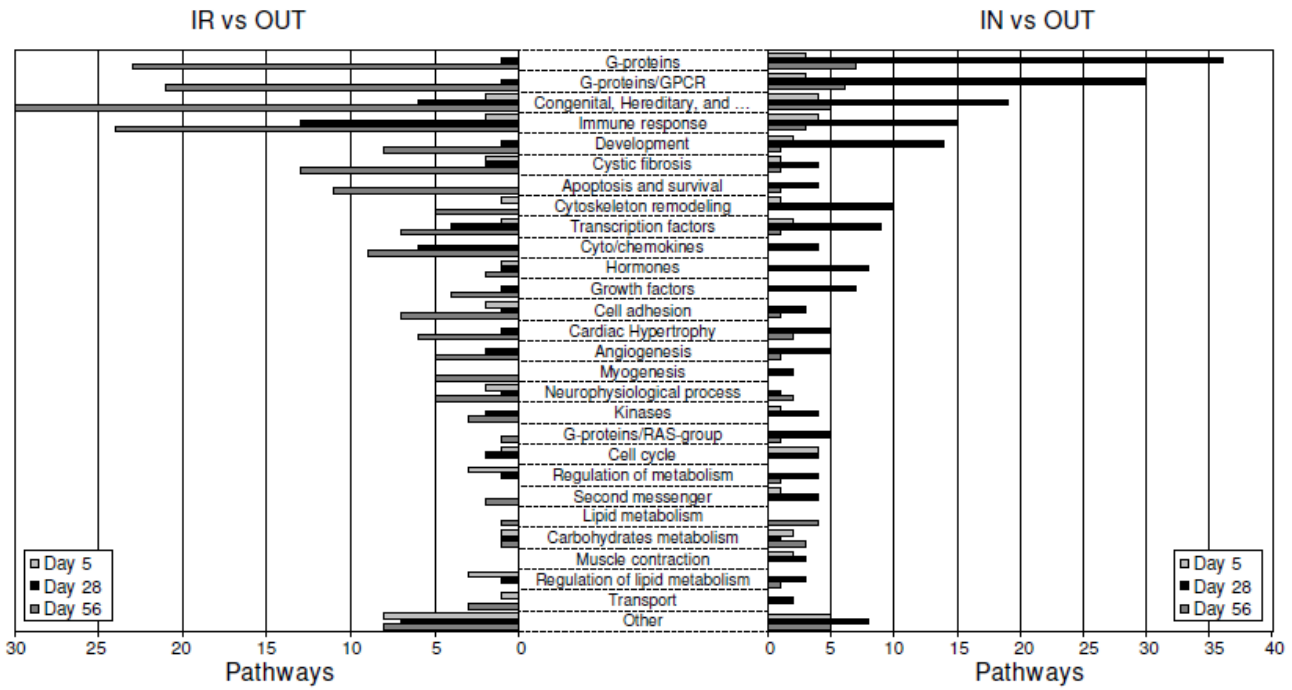

Figure 7
MetaCore pathway analysis of differentially expressed genes of animals housed in different environments. Differentially expressed genes ($P < 0.05$) were imported into GeneGo MetaCore analytical software to determine significantly enriched canonical pathways in each group. Data represent the distribution in cell process categories of statistically significantly enriched pathways ($P < 0.05$) of the comparisons IR vs OUT **(A)** and IN vs OUT **(B)**. Most pathways from both comparisons group into five categories: G-proteins; G-protein coupled receptor; congenital, hereditary and neonatal diseases and abnormalities; immune response; and development. Note that there is redundancy in category allocation.

Mulder et al., 2009

Microflora and the immune system

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Microbes regulate innate and adaptive immunity

Innate immune system

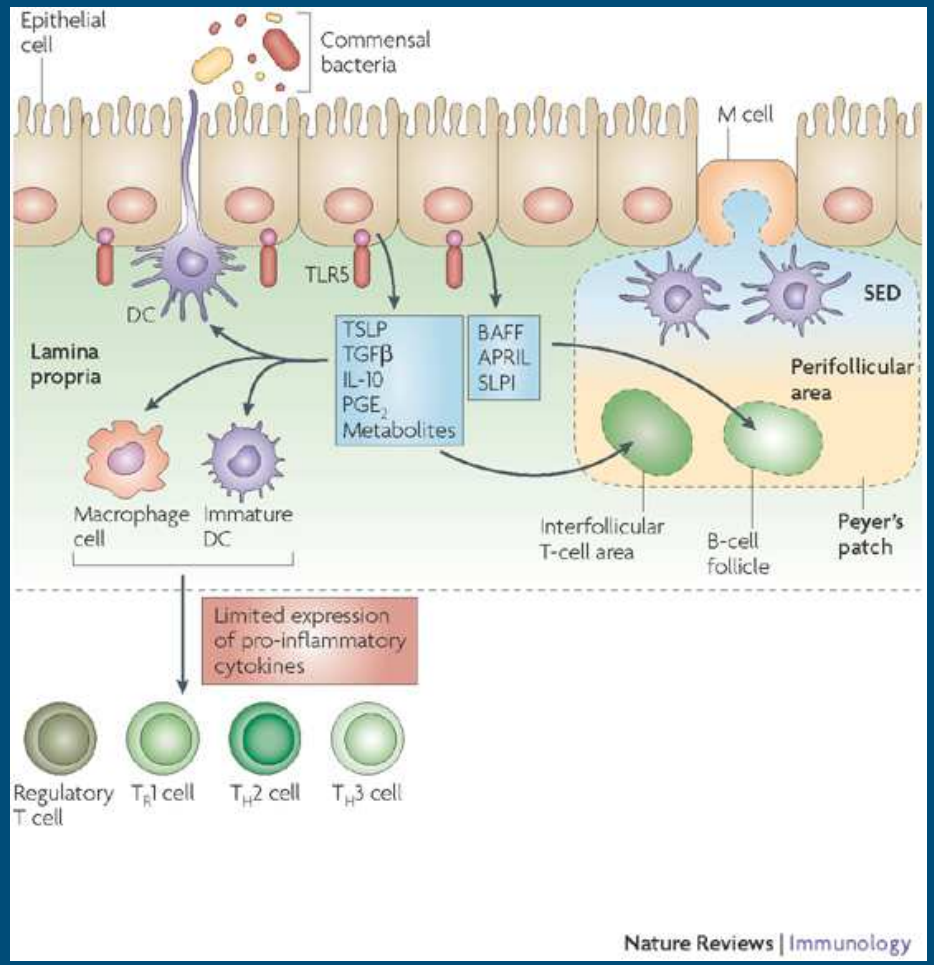
- A universal and evolutionarily conserved system of host defence
- Induced upon infection with microbes
- Based on recognition of pathogen associated molecular patterns (PAMPS)
- Main players include macrophages, monocytes, neutrophils and dendritic cells
- Plays a fundamental role in the induction and regulation of the adaptive immune response

Adaptive immune system

- Induced upon infection by specialized pathogens
- Based on recognition of specific antigens presented by antigen presenting cells (e.g. dendritic cells)
- Carried out by effector cells (T- and B-cells)

Kelly, 2009

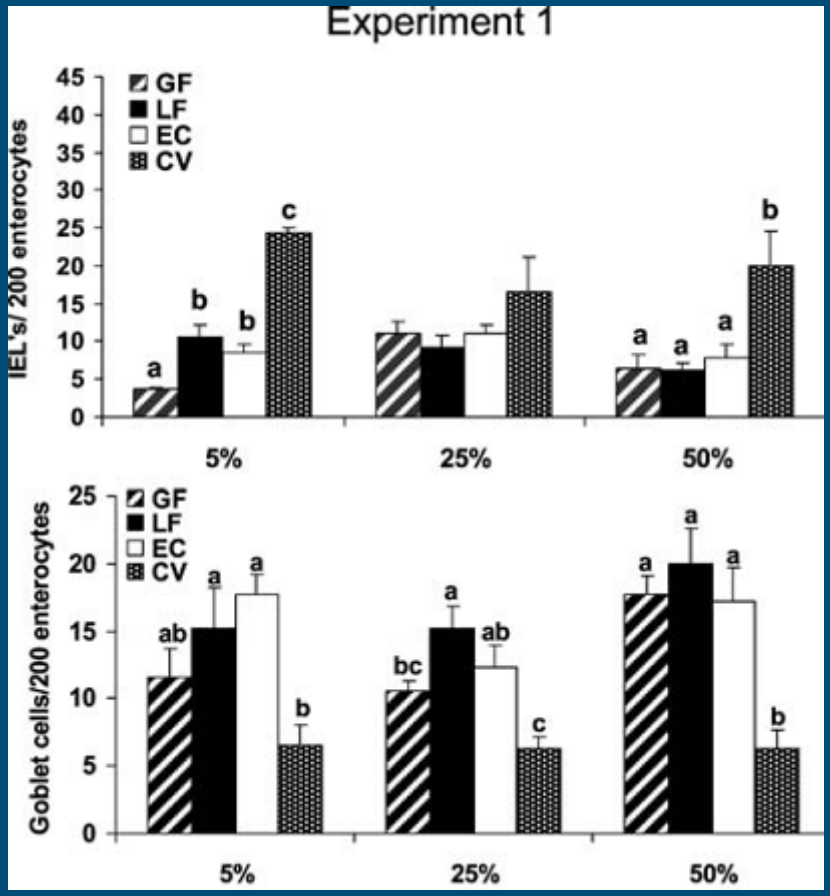
Commensal bacteria and gut immune function



Basal recognition of commensal bacteria by intestinal epithelial cells (IECs) may influence the secretion of cytokines that can directly influence the expression of pro-inflammatory cytokines by dendritic cell (DC) and macrophage populations that resident in the lamina propria and Peyer's patches. Signals derived from commensal bacteria may influence tissue-specific 'licensing' of accessory-cell functions resulting in the expansion and/or survival of T cells with regulatory capacities, including regulatory T cells. In addition other metabolites have the capacity to directly regulate the functions of both antigen-presenting cells and lymphocytes in the intestinal microenvironment.

Arts, 2008

Number of intraepithelial lymphocytes (IELs) and globet cells as affected by intestinal microflora

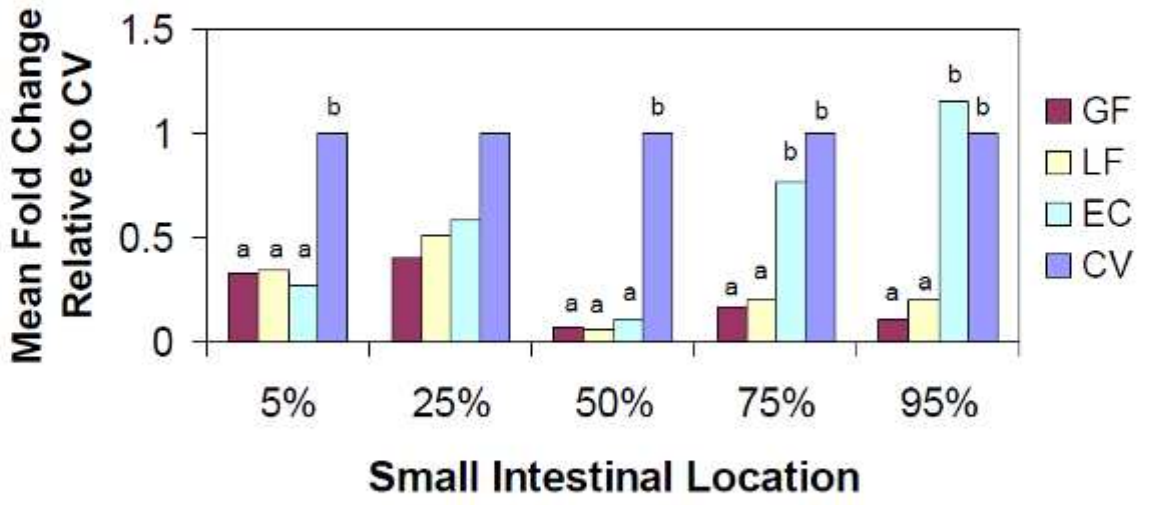


Small intestinal location

Shirkey et al., 2006

Pro-inflammatory cytokine expression

IL-1β



UNIVERSITY OF SASKATCHEWAN

Shirkey et al. 2006

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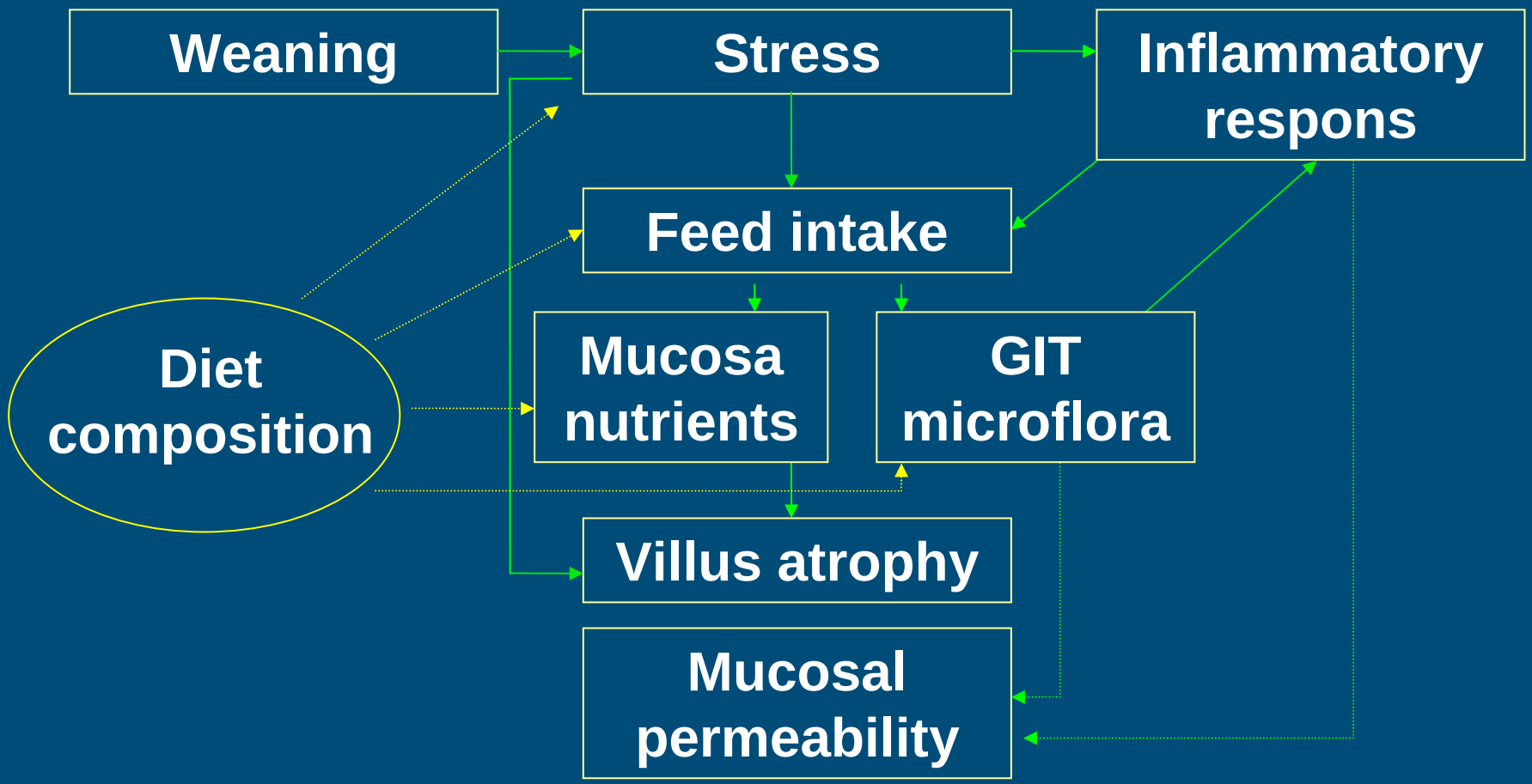
Effects of weaning



Microbial composition in different parts of the GI tract of weaned piglets

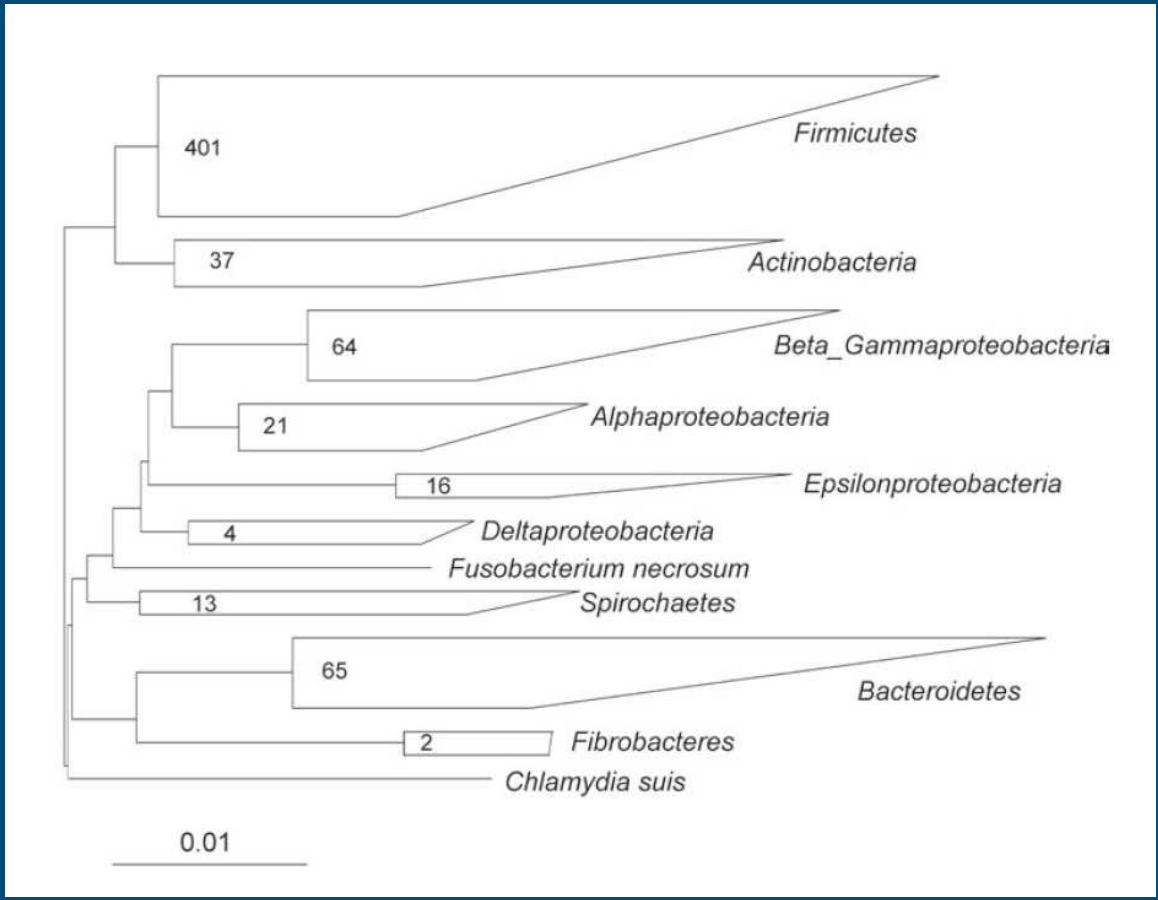
Table 4. Quantitative real-time PCR analysis of total bacteria, lactobacilli, *Lactobacillus sobrius* and *Streptococcus suis* in digesta samples of porcine stomach, jejunum and ileum

	Stomach		Jejunum		Ileum	
Species	Day 21	Day 24	Day 21	Day 24	Day 21	Day 24
Bacteria	5.40 ± 0.07	7.95 ± 0.61*	8.99 ± 0.44	8.23 ± 0.84	9.72 ± 0.19	8.29 ± 0.77*
Lactobacilli	4.55 ± 0.13	6.04 ± 0.54*	8.11 ± 0.30	5.60 ± 0.20*	9.29 ± 0.20	6.80 ± 0.91*
Lactobacilli/bacteria (%)	14.13	1.23	13.18	0.23	37.15	3.24
L. sobrius	4.14 ± 0.25	5.64 ± 0.15*	7.12 ± 1.01	5.05 ± 0.14*	8.65 ± 0.63	5.94 ± 1.03*
L. sobrius/bacteria (%)	5.50	0.49	1.35	0.07	8.51	0.45
S. suis	< 4	7.36 ± 1.06*	6.34 ± 0.97	7.04 ± 0.61	7.24 ± 0.29	7.60 ± 0.82
S. suis/bacteria (%)	< 3.98	25.70	0.22	6.46	0.33	20.42

*Significant differences between day 21 and day 24 compared at $P < 0.05$.
Three samples were quantified for each age and counts are expressed as mean ± SD Log 10 (16S rRNA gene copies g^{-1} wet weight), $n = 3$.

Su et al. 2008

SSU rRNA-based phylogenetic tree of the unique phylotypes found in the pig gastrointestinal tract - PITChip). Numbers of distinct phylotypes are given for each phylum (total 627 phylotypes).



Pérez Gutiérrez et al., 2010

Dietary organic acids and gut microbial composition in piglets three weeks post weaning

Higher taxonomic Group	Group	Distal Ileum		Proximal Colon	
		p-value		p-value	
		(OA1)	(OA2)	(OA1)	(OA2)
Bacilli	*Lactobacillus gasseri*-like	0.628	0.292	0.414	**0.022**
Bacteroidetes	*Alistipes*-like	**0.005**	0.060	0.130	0.146
	Bacteroides coprosuis-like	0.278	**0.032**	0.471	0.285
	Bacteroides distasonis-like	0.239	**0.014**	0.821	0.518
	Bacteroides fragilis-like	0.188	**0.001**	0.760	0.551
	Bacteroides pyogenes-like	0.253	**0.025**	0.858	0.585
	Bacteroides vulgatus-like	0.252	**0.012**	0.748	0.508
	Paludibacter propionicigenes-like	0.252	**0.011**	0.707	0.485
	Prevotella ruminicola-like	0.265	**0.039**	0.637	0.388
	Uncultured *Bacteroidetes*	0.207	**0.013**	0.628	0.568
	Uncultured *Porphyromonadaceae*	0.226	**0.033**	0.575	0.382
	Uncultured *Prevotella*	0.247	**0.003**	0.505	0.375
Betaproteobacteria	Uncultured *Betaproteobacteria*	**0.050**	0.955	0.975	0.840
Clostridium cluster IX	*Mitsuokella multiacida*-like	0.122	0.332	0.271	**0.015**
Deferribacteres	*Mucispirillum schaedleri*-like	0.279	**0.032**	0.469	0.279
Epsilonproteobacteria	*Campylobacter*	0.072	0.106	**0.041**	0.100
	Helicobacter	**0.015**	0.085	0.172	0.631
Flavobacteria	*Chryseobacterium*-like	0.572	0.705	**0.024**	0.503
Gammaproteobacteria	*Actinobacillus*-like	0.079	0.842	0.383	**0.021**
Sphingobacteria	*Sphingobacterium thalpophilum*-like	**0.009**	**0.023**	**0.050**	0.383
Spirochaetes	*Treponema*-like	**0.040**	0.269	0.340	0.177

Pre- and probiotics

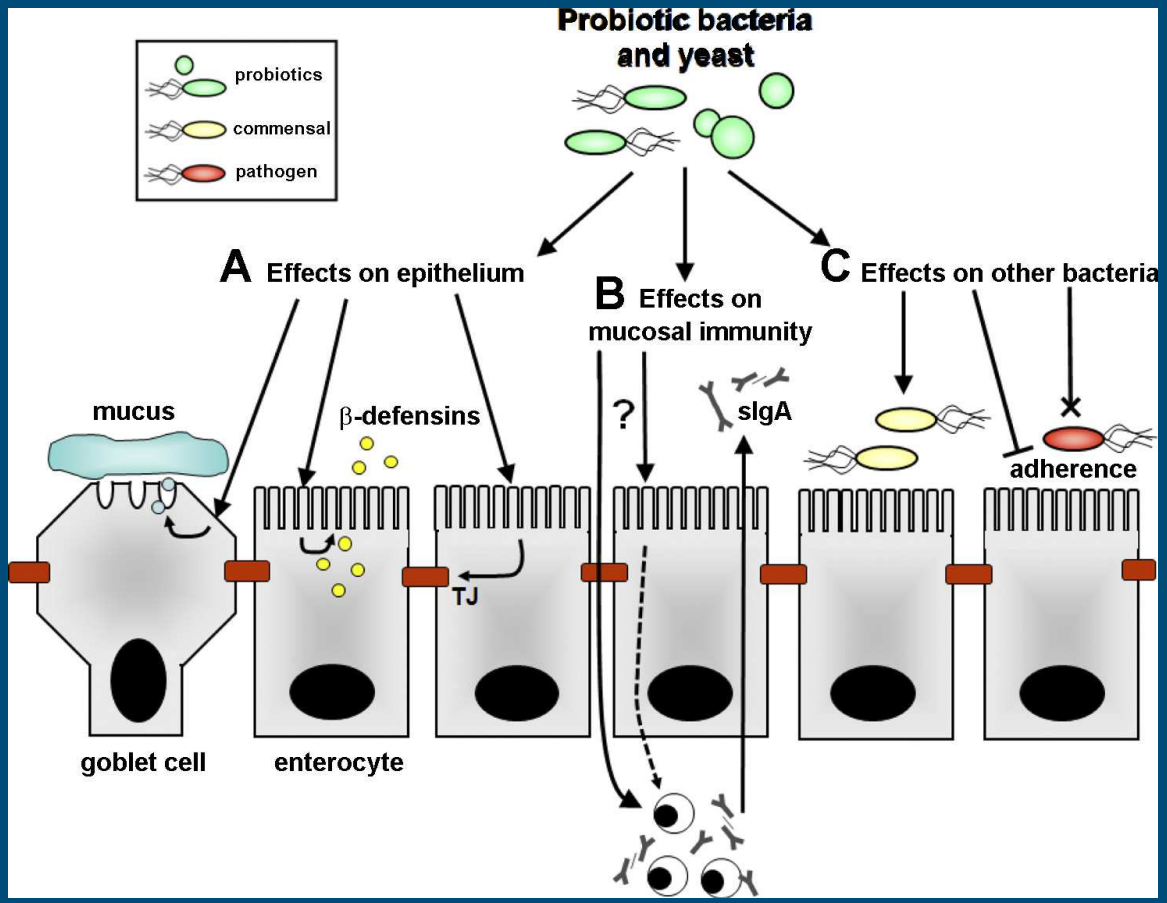
Substrate

Live micro-organisms

- Modify intestinal microbiota
- Increase production of volatile fatty acids (VFA)
- Stimulate the immune system
- Reduce inflammatory reactions
- Increase B vitamin synthesis
- Prevent pathogen colonization
- Improve mineral absorption
- Enhance animal performance
- Decrease ammonia and urea excretion

Patterson and Burkholder, 2003

Effects of probiotics in the small intestine



Ohland & MacNaughton, 2010

Phytobiotic feed additives

Plant extracts

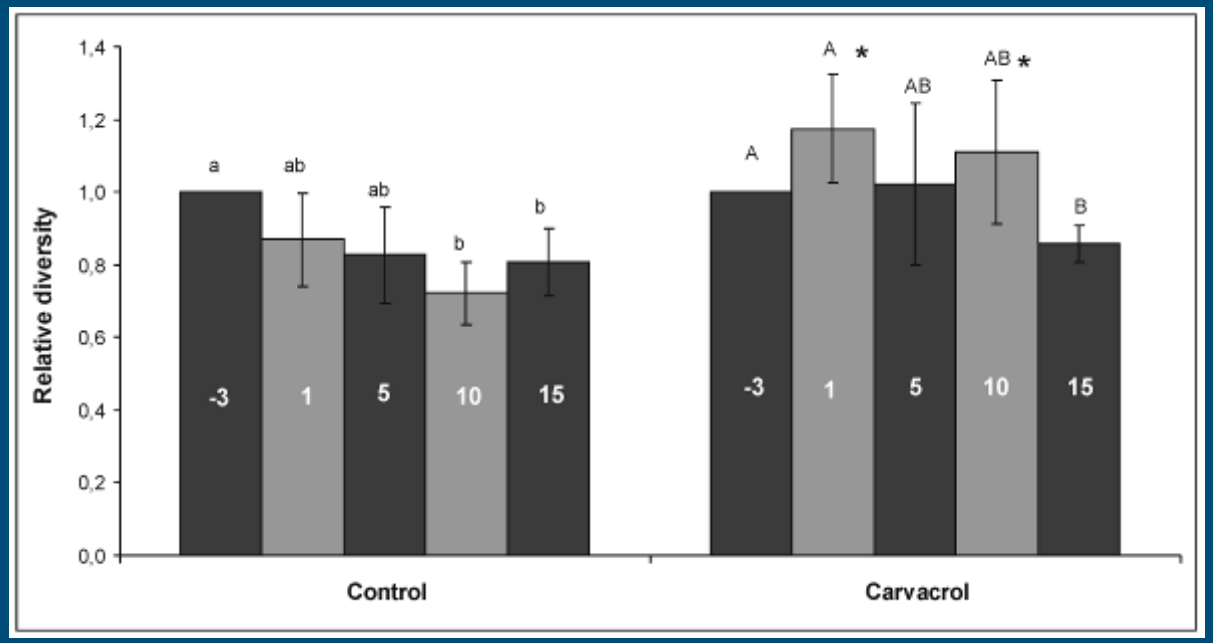
- Oregano
- Rosemary
- Carvacrol
- Cinnamaldehyde
- Yucca extract

Essential oil blends

Herbs and spices

- Thyme
- Garlic
- Herb mixes

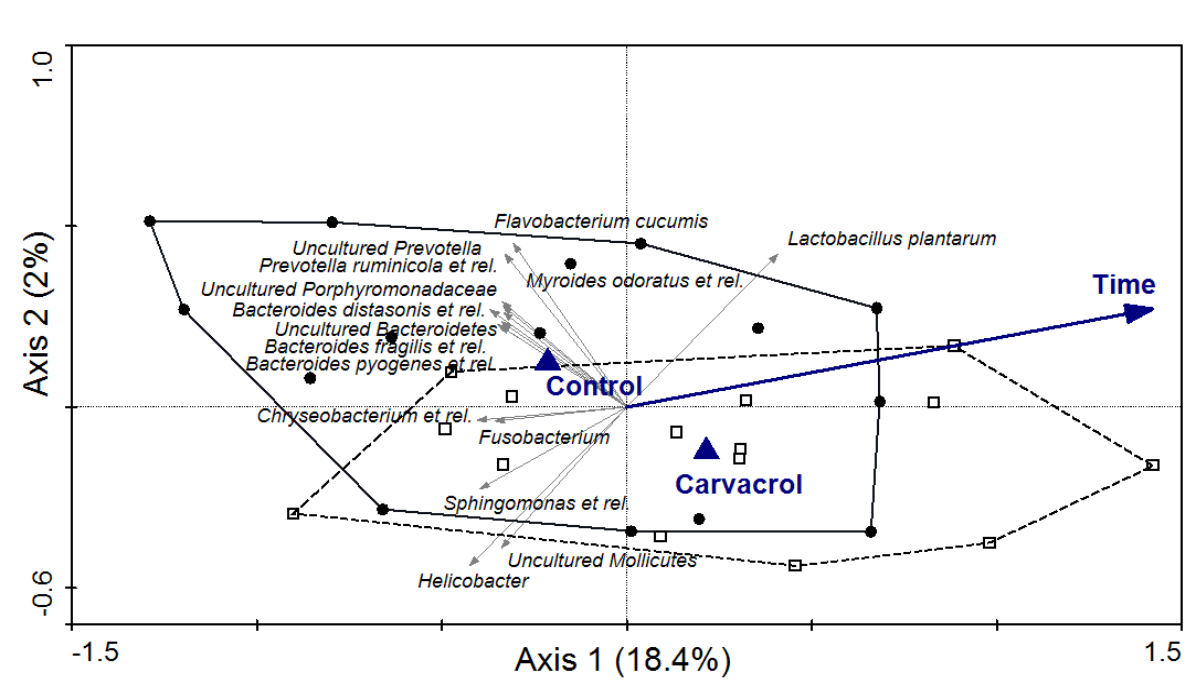
Relative diversity of PITChip microbial profiles



Relative diversity of PITChip microbial profiles along the sampling period represented the average diversity for both the control group and the carvacrol group. Time points are labeled in the interior of the bars. ∗ indicates that the difference between the treatment and the carvacrol is significant and letters indicate significant differences between time points (small for the control and capital for carvacrol), $p < 0.05$. Bars represent means and SEM.

Pérez Gutiérrez et al, 2010

PITChip analysis of ileal digesta in piglets



Redundancy analysis triplot of the ileum microbiota composition, as measured by PITChip analysis and expressed by the summed hybridization signals of 144 phylogenetic groups for 15 pigs from the control group (•) and 15 pigs from the carvacrol treated group (□). Microbial groups that contributed at least 20% to the explanatory axes are represented as vectors. The environmental nominal variables Control and Carvacrol are centroids of the plot and the non-nominal variable time is represented as a vector, the length of which corresponds to variance that can be explained by the environmental variable. Monte Carlo permutation test indicated that changes in microbial profiles are significantly correlated to Time ($p < 0.01$).

Pérez Gutiérrez et al., 2010

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SEVENTH FRAMEWORK PROGRAMME
THEME 2

Food, Agriculture and Fisheries, and Biotechnology

Project acronym **INTERPLAY**

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

LIVESTOCK RESEARCH
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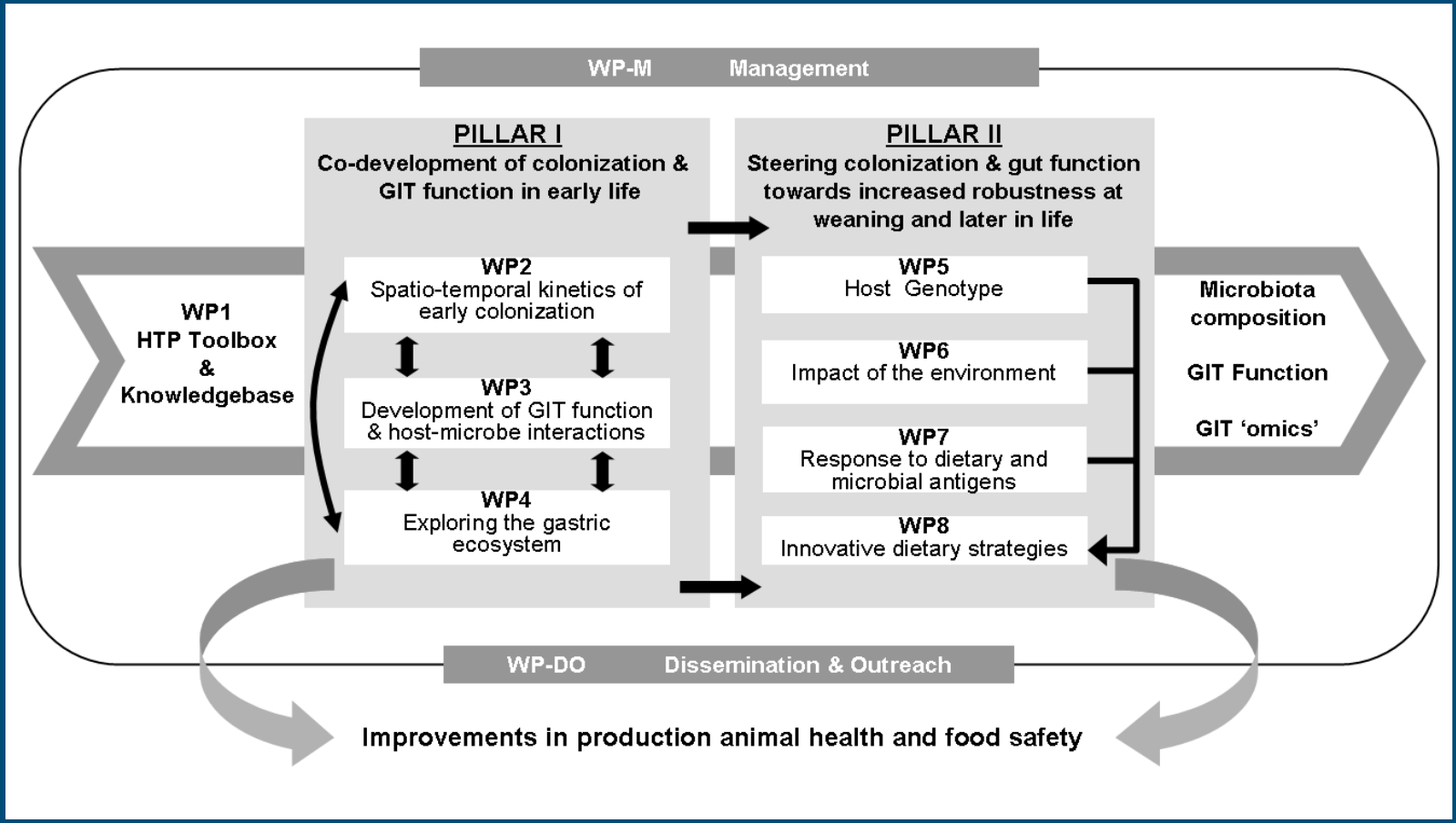
Scientific objectives (1)

- Generate a knowledgebase on the kinetics of colonisation by commensal as well as potentially pathogenic microbiota along the GIT of young pigs, and concomitant impact on GIT microbiota composition as well as gastro-intestinal function throughout life
- Provide understanding at the cellular level of host-microbe interactions that drive gut function development
- Identify the extend to which the sow influences the microbial colonisation process, and concomitantly gut function in the offspring, either directly through genotype or through the sows own microbiota

Scientific objectives (2)

- Expand our knowledge on the short- and long term effects of antibiotic treatment early in life on microbiota composition and gut function throughout life
- Provide novel insight in the effect of the rearing environment, including the farm hygienic status, on the development of microbiota and gut function
- Categorise the impact of innovative pre- and probiotic treatments on the co-development of microbiota colonization and gut function
- Generate novel hypotheses and leads towards the rational design of management strategies for improved farm animal health and robustness

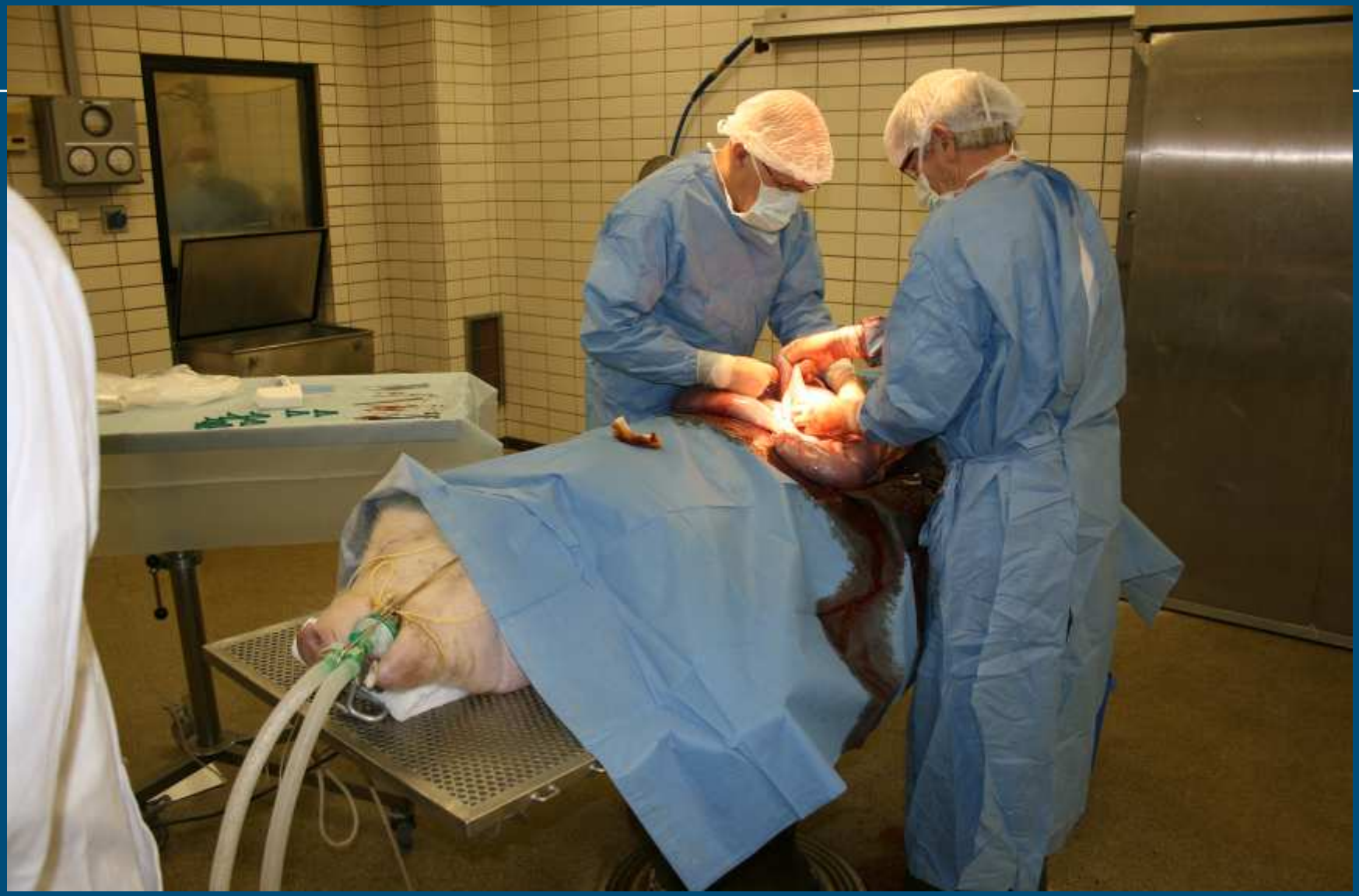
Project components EU Interplay



Development of a model to create piglets varying in gut microbial colonization

To compare two groups of CDCD piglets:

- Group 1, exposed to Lactobacillus sobrius, Clostridium glycolicum, Parabacteroides spp “Bristol mix” on day 1, 2 and 3 and a diverse microflora (feces from a conventional donor sow) on day 3 and 4 – “low sanitary status”
- Group 2, only exposed to Lactobacillus sobrius, Clostridium glycolicum, Parabacteroides spp “Bristol mix” on day 1, 2 and 3: “high sanitary status”



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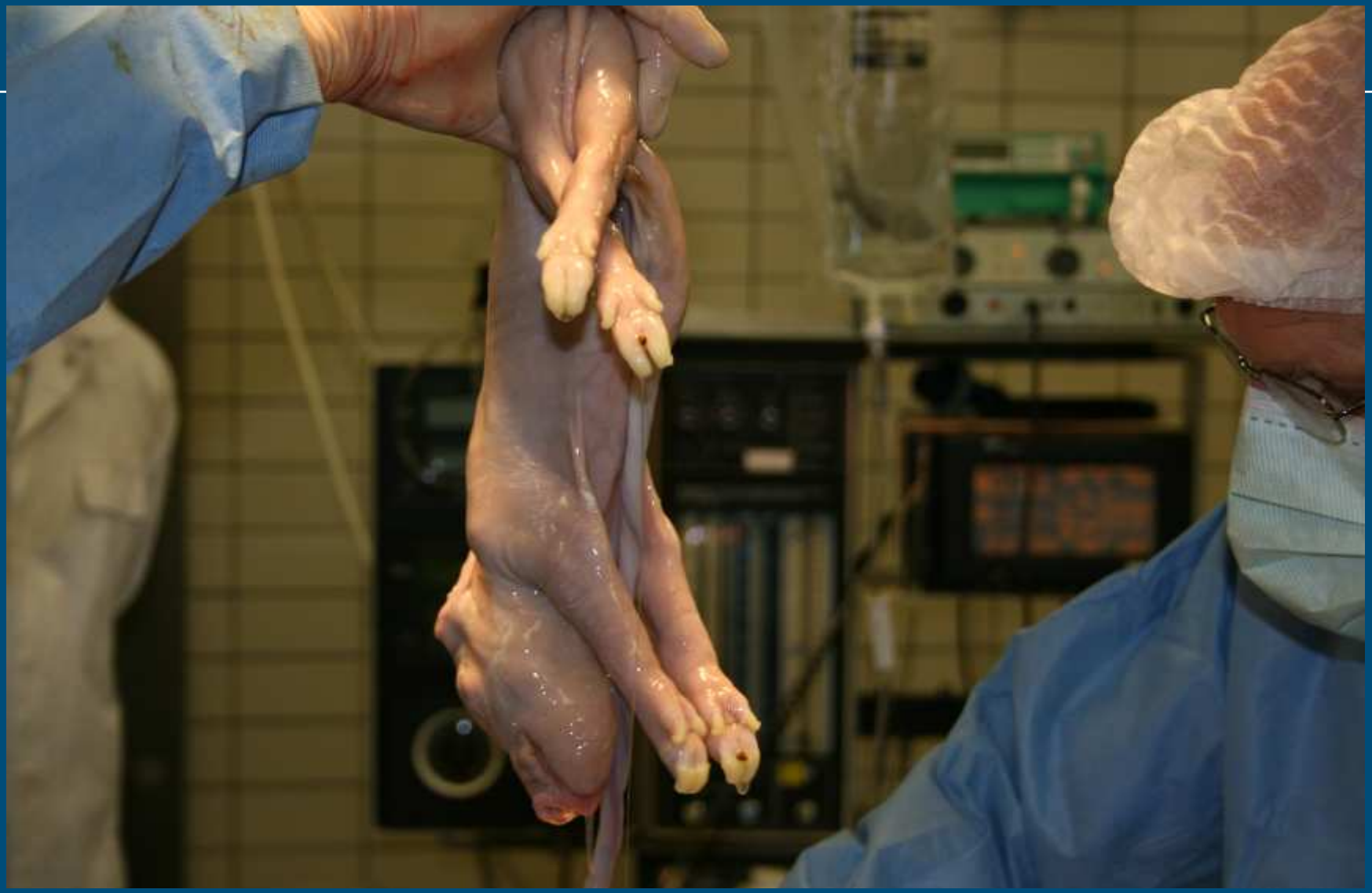

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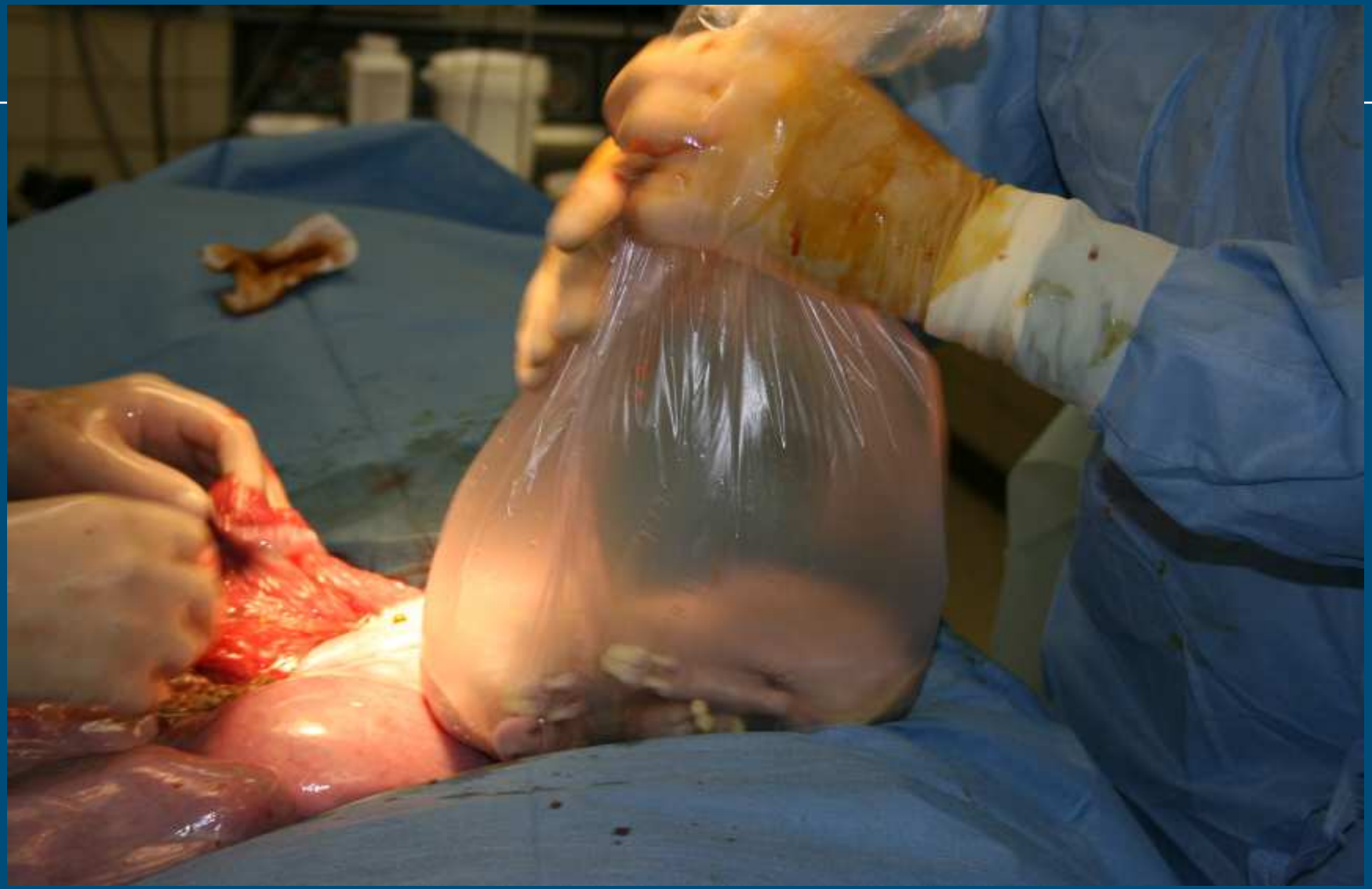

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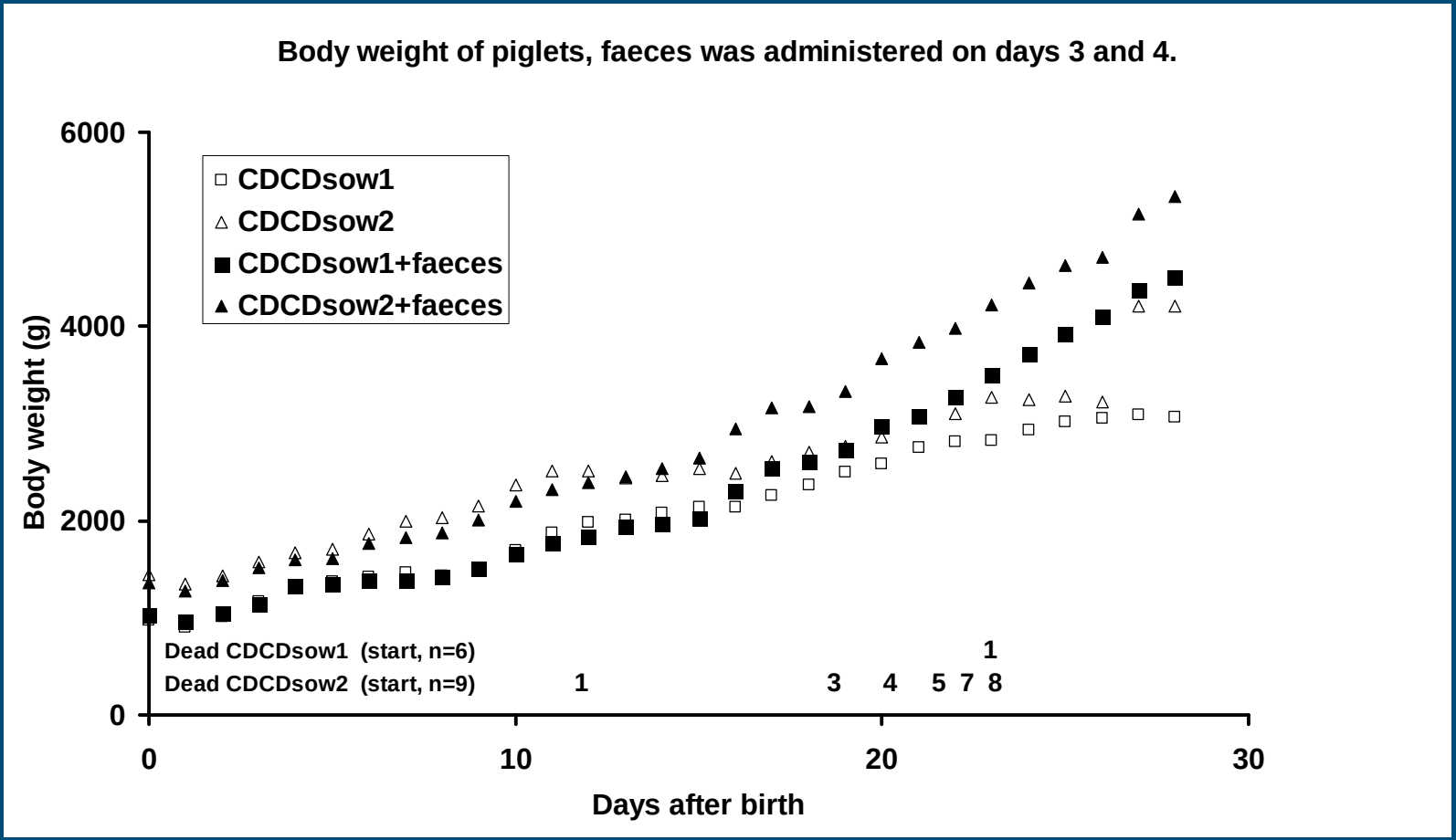
Development of a model to create piglets varying in gut microbial colonization



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Conclusions

- Microflora in the gastro-intestinal tract of pigs throughout their life plays a multifunctional role in maintaining health, influencing the local immune system and gut development.
- Significant progress has been made in the characterisation of microbiota in the GI tract.
- Diet composition and feed additives can be used to influence intestinal microbial composition.
- More emphasis should be given to the early colonisation of the GI tract and to the possibilities to actively influence colonisation thereby improving gut health and development.

Thank you for your attention

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