Maternal dietary interventions affect piglet intestinal development in different ways

Effects on mucosal gene expression and microbiome

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Feed4 Foodure

EAAP, Belfast, September 2016

session 72 "Optimising animal nutrition at an integral level: improving health, nutrient use efficiency and product quality“

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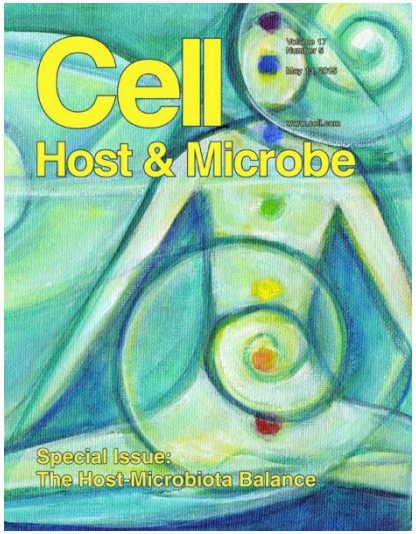
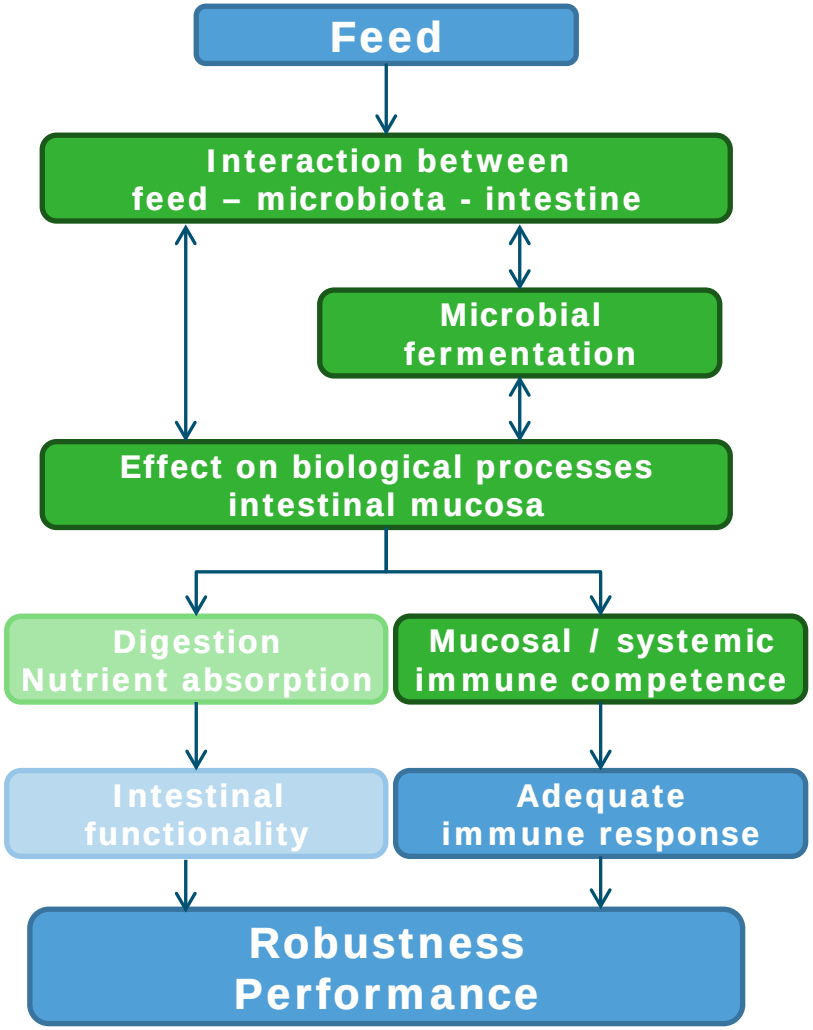
Background

- Feed4Foodure is a large public private partnership with Dutch feed industry
- Goal is to determine immune competence of livestock after dietary interventions



- Immune competence is the potential to adequately respond to stimuli
- Immune competence is determined by Feed – Microbiota – Host interaction



Immune competence development

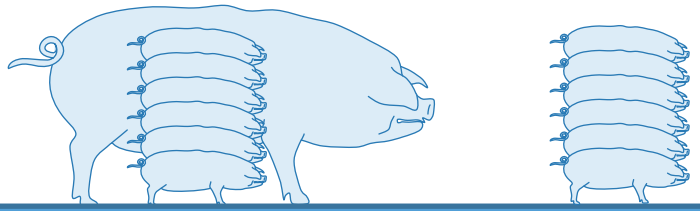
- First colonization of intestine starts at birth; (vaginal) birth, breast-feeding, antibiotic use and weaning important factors for microbial colonization (Bäckhed *et al.*, 2015 Cell Host & Microbe **17**:690)
- Maturation of mucosal immunity and epithelial barrier function parallels microbiota development in time; intestinal microbiota teaches the mucosal immunity (Vangay *et al.*, 2015 Cell Host & Microbe **17**:553 & Kabat *et al.*, 2014 Trends Immunol. **35**(11):507)
- In mammals weaning & introduction of solid feed greatly affect microbiota composition and diversity (Bian *et al.*, 2016 Environm. Microbiol. **18**(5):1566)
- Early life events are major factors influencing later life microbiota composition and diversity; birth and weaning seam to be time of particular susceptibility to changes (Thompson *et al.*, 2008 ISME J **2**:739 & Mulder *et al.* 2011 PLOS One **6-**12)

➔ To influence immune competence using dietary interventions, dietary changes have to be applied at young age, either neonatal or maternal

Experimental set-up

GOAL: Determine effect of 3 maternal feed interventions on intestinal development and immune competence of offspring piglets

- Medium Chain Fatty acids (0.1% C10 / 0.1% C12 in feed): Probably affects microbiota composition proximal intestine
- Beta-glucan (0.1% Macrogard) in feed: Immunomodulatory effects
- Galacto-oligosaccharides (0.17% Vivinal GOS in feed): beneficial effects on humans → bifidogenic effect colon
- Control



Dietary intervention on sows from 1 week before gestation until weaning

Day 1 – 31:
Performance

Day 1 & day 31 (3 days post weaning):
- Intestinal gene expression
- Microbiota composition & diversity
- Intestinal morphology

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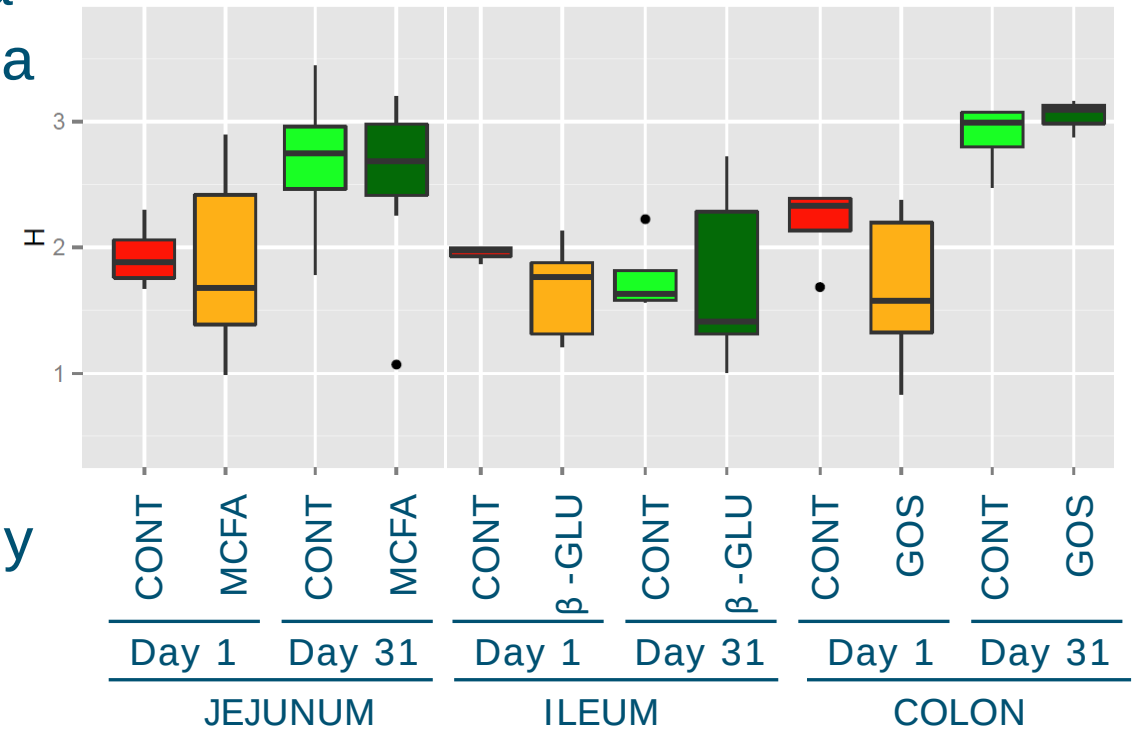
Effect diets on microbiota composition

- Determine microbiota composition in digesta using community scale 16S RNA sequencing at day 1 and day 31 (3d post weaning)
- Increase in α-diversity (Shannon) over time
- No differences in diversity due to treatment



MCFA have strongest effect on microbiota

- Number of statistical changes in microbiota composition on family level

Intervention	Day	Number of statistically significant differences compared to control
MCFA (jejunum)	1	45
	31	19
β-glucan (ileum)	1	2
	31	0
GOS (colon)	1	5
	31	0

- Minor changes due to β-glucans and GOS
- MCFA induce changes in composition
  - Strongest effect on day 1, early life colonization
  - Suggestive for transmission effect via other routes than milk

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Mucosal response to dietary interventions

- Determine gene expression in intestinal scrapings of offspring piglets using whole genome microarrays

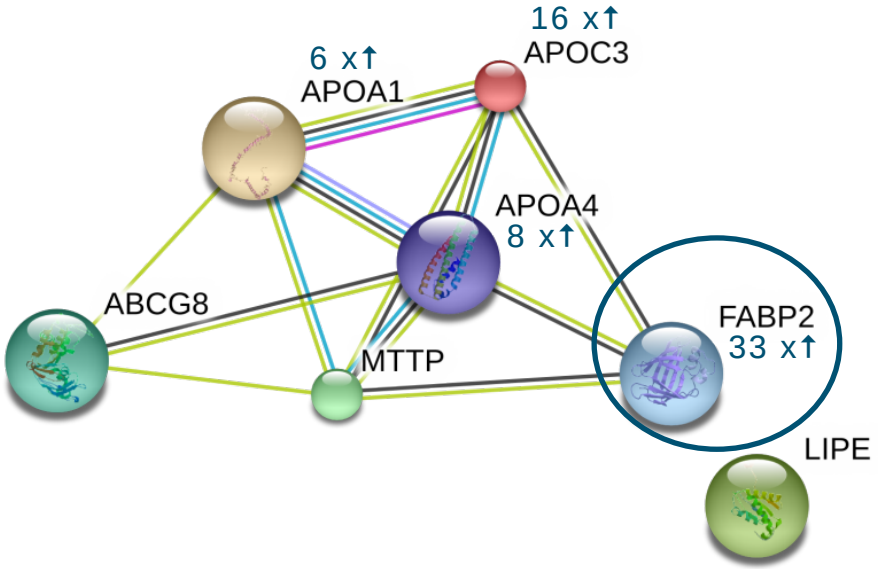
Intervention	Day	Number of regulated & annotated genes compared to control*
MCFA (jejunum)	1	3
	31	203
β-glucan (ileum)	1	0
	31	302
GOS (colon)	1	2721
	31	878

* FC > 2; p ≤ 0.05

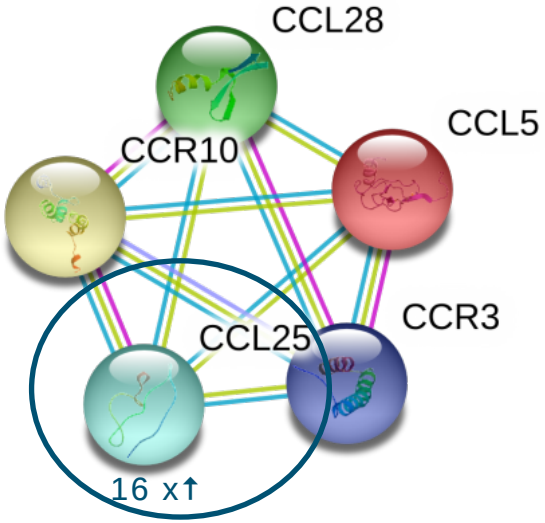
- All dietary interventions strongly affect intestinal gene expression in offspring piglets
- GOS has large effect on Day 1; β-glucans and MCFAs have main effect on Day 31
  - Transmission putatively in part via milk

Major processes affected by MCFA @day31

Fat Metabolism



Chemokine signalling



FABP2 (33 x ↑)

- Energy homeostasis
- Fatty Acid Binding Protein

CCL25 (16 x ↑)

- Antimicrobial
- Chemotactic for macrophages
- Role in T-cell development

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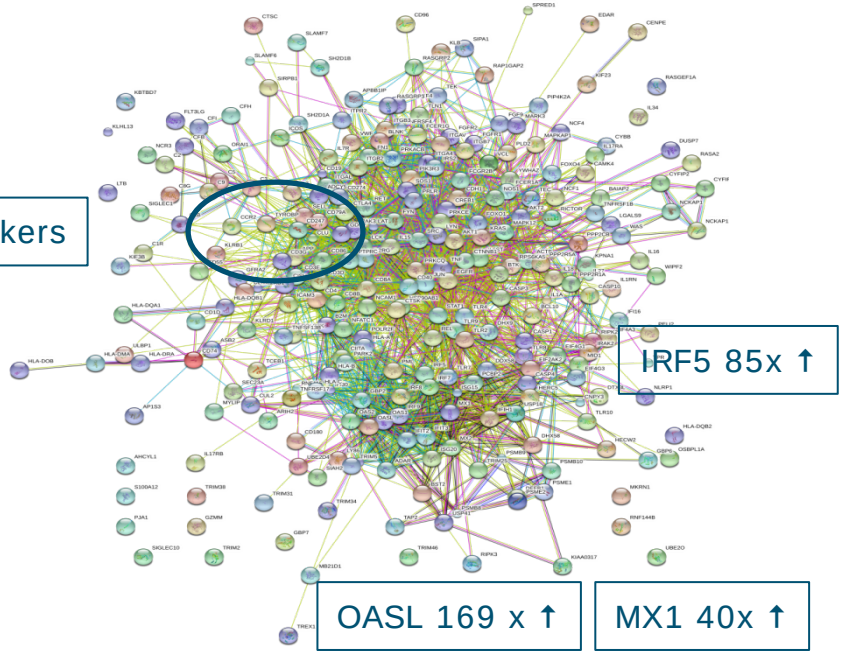
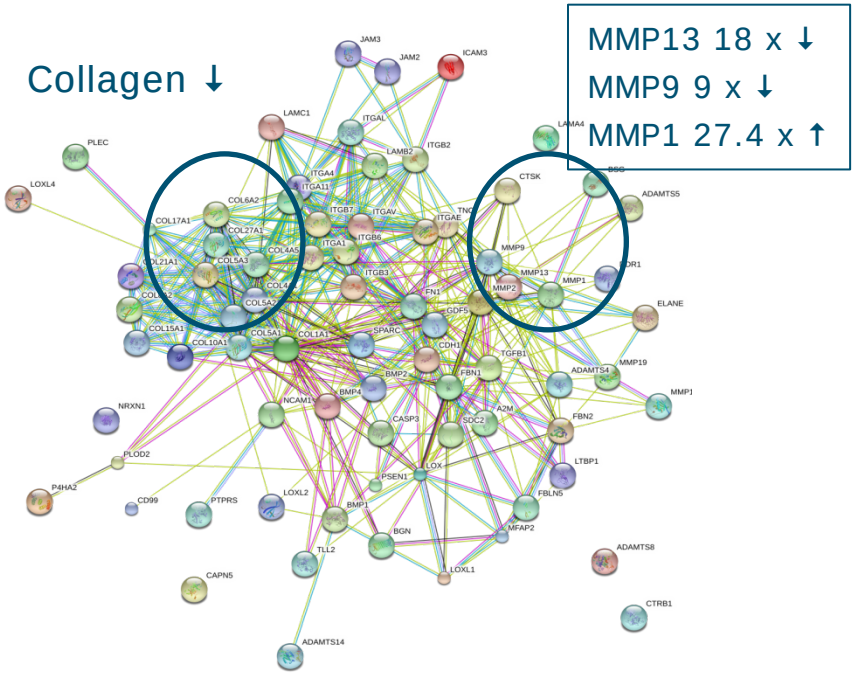
Major processes affected by GOS @day1

Immune processes



- Strong antiviral response induced by IFN-γ
- Also induced by Lactococcus

Extracellular matrix



- Collagen production down regulated
- MMP1 cleaves collagen = upregulated
- MMP13 / MMP9 degrade ECM = downregulated

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Conclusion / discussion (1)

- Maternal dietary interventions have a significant impact on intestinal development of new-born piglets
  - Mucosal gene expression changes
  - Local microbiota affected
  - Effect are measured in a critical period for later life immunity and microbiome; this might contribute to efficiency of digestive processes
- Large differences in intestinal development of new-born piglets between the 3 maternal dietary interventions
- Effects of GOS > effect MCFA > effect β-glucans

Follow-up / discussion

- Responsible components and routes of transgenerational effects are unknown
  - Tranmission route via milk is candidate → further analyses are ongoing
- Long term effects on metabolism and immune competence are unknown
  - Requires performance and challenge studies respectively
- Functional validation of gene expression data
  - Immunohistochemistry / metabolic analyses planned
- Similar dietary interventions directly administered to newborn piglets neonatally result in different effects (preliminary data)
  - Administration route important

Central Veterinary Institute

- Mari Smits
- Stéphanie Vastenhouw
- Alex Bossers
- Freddy de Bree
- Frank Harders
- Ralph Kok

Trouw Nutrition

- Janneke Allaart
- Carlijn de Bruijn
- Hubèrt van Hees
- Petra Roubos

Wageningen Livestock Research

- Dirkjan Schokker
- Carola van der Peet
- Annemarie Rebel

Financial support

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Conclusion / discussion (2)

- Maternal administration of MCFA affects the offspring piglets:
  - Functional changes in intestinal fat metabolism (major effects)
  - Local immunological development (minor effect)
  - Early life microbial colonization
  - Effect putatively transmitted via milk?
- Maternal administration of GOS affects the offspring piglets:
  - Local immunological development (major effect)
  - Extracellular matrix (barrier function?) (major effect)
  - Minor changes in microbiota
  - Effect probably not transmitted via milk?
- Maternal administration of β-glucans affects the offspring piglets by moderate but diffuse changes in gene expression and almost no effect on microbiota (difficult to interpret)

Statistically significant differences @ Family Level: Day 1 – Maternal administration

Beta-glucans - Ileum

	p.value	FDR
k__Bacteria.p__Firmicutes.c__Bacilli.f__Turicibacteraceae	0.006060606	0.775757576
k__Bacteria.p__Firmicutes.c__Bacilli.f__Staphylococcaceae	0.024242424	0.785548921

GOS - Colon

	p.value	FDR
k__Bacteria.p__Actinobacteria.c__Actinobacteria.Other	0.015873016	0.574169963
k__Bacteria.p__Actinobacteria.c__Actinobacteria.f__Streptomycetaceae	0.015873016	0.574169963
k__Bacteria.p__Firmicutes.c__Bacilli.f__Turicibacteraceae	0.015873016	0.574169963
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.f__Aeromonadaceae	0.017942811	0.574169963
k__Bacteria.p__Firmicutes.c__Clostridia.f__EtOH8	0.041620063	0.774685231

Statistically significant differences @ Family Level: Day 31 – Maternal administration

MCFA - Jejunum

Taxon	p.value	FDR
k__Bacteria.p__Acidobacteria.c__Holophagae.f__Holophagaceae	0.003127609	0.295454545
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.f__Legionellaceae	0.003127609	0.295454545
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.f__Cryomorphaceae	0.005400041	0.295454545
k__Bacteria.p__Firmicutes.c__Bacilli.f__Turicibacteraceae	0.006060606	0.295454545
k__Bacteria.p__Bacteroidetes.c__Cytophagia.f__Cytophagaceae	0.010030543	0.351744471
k__Bacteria.p__Proteobacteria.c__Betaproteobacteria.f__Methylophilaceae	0.013227748	0.351744471
k__Bacteria.p__Bacteroidetes.c__Sphingobacteriia.f__	0.01623436	0.351744471
k__Bacteria.p__OD1.c__ZB2.f__	0.01623436	0.351744471
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.f__Syntrophobacteraceae	0.01623436	0.351744471
k__Bacteria.p__Proteobacteria.c__Betaproteobacteria.f__Rhodocyclaceae	0.024242424	0.393939394
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.Other	0.026118917	0.393939394
k__Bacteria.p__Firmicutes.c__Bacilli.f__Thermoactinomycetaceae	0.028283862	0.393939394
k__Archaea.p__Euryarchaeota.c__Methanobacteria.f__Methanobacteriaceae	0.029387286	0.393939394
k__Bacteria.p__Deferribacteres.c__Deferribacteres.f__Deferribacteraceae	0.033359945	0.393939394

Statistically significant differences @ Family Level: Day 31 – Neonatal administration

MCFA - Jejunum

	p.value	FDR
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.f__Methylobacteriaceae	0.022644974	0.660962567
k__Bacteria.p__Actinobacteria.c__Actinobacteria.f__Nocardioidaceae	0.028283862	0.660962567
k__Bacteria.p__Firmicutes.c__Bacilli.f__Paenibacillaceae	0.028283862	0.660962567
k__Bacteria.p__Proteobacteria.c__Betaproteobacteria.f__Rhodocyclaceae	0.028283862	0.660962567
k__Bacteria.p__Firmicutes.c__Bacilli.f__Bacillaceae	0.029387286	0.660962567
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.f__Halomonadaceae	0.042424242	0.660962567

Beta glucans - Ileum

k__Bacteria.p__Proteobacteria.c__Betaproteobacteria.f__Neisseriaceae	0.024242424	0.97536339
k__Bacteria.p__SR1.c__.f__	0.037418757	0.97536339
k__Bacteria.p__Bacteroidetes.c__Bacteroidia.f__RF16	0.042424242	0.97536339

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1. Microbiota drives establishment of the architecture of the mucosal immune system.
2. Different patterns of colonisation, occuring as a consequence of different rearing environments, drive development of different mucosal immune systems
3. This can be manipulated in early life by interventions which modulate microbiota/immunity such as diet and/or probiotic (and/or prebiotic)
4. Birth and weaning seem to be times of particular susceptibility to changes.

1. However, the patterns of change are complex, interact, and are affected by multiple, as yet undefined, factors.
2. Conclusions based on empirical observations are only right under the conditions that were tested
3. Without clear understanding of mechanisms, the effects of any specific intervention on specific farms will be hard to predict.