Maternal dietary interventions affect piglet intestinal development in different ways

Effects on mucosal gene expression and microbiome


astrid.degreeff@wur.nl

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, breastfeeding, 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
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

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Day</th>
<th>Number of statistically significant differences compared to control</th>
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<tr>
<td>MCFA (jejunum)</td>
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- 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
Mucosal response to dietary interventions

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

<table>
<thead>
<tr>
<th>Intervention</th>
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* 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**

- FABP2 (33 x ↑)
  - Energy homeostasis
  - Fatty Acid Binding Protein

**Chemokine signalling**

- CCL25 (16 x ↑)
  - Antimicrobial
  - Chemotactic for macrophages
  - Role in T-cell development
Major processes affected by GOS @day1

**Immune processes**
- Strong antiviral response induced by IFN-γ
- Also induced by Lactococcus

**Extracellular matrix**
- Collagen production downregulated
- MMP1 cleaves collagen = upregulated
- MMP13 / MMP9 degrade ECM = downregulated

CD markers:
- OASL 169x ↑
- MX1 40x ↑
- IRF5 85x ↑

MMP13 18x ↓
MMP9 9x ↓
MMP1 27.4x ↑
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
  - Transmission 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
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**

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**GOS - Colon**

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

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

**MCFA - Jejunum**

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**Beta glucans - Ileum**

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1. Microbiota drives establishment of the architecture of the mucosal immune system.

2. Different patterns of colonisation, occurring 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.