EFFICIENT & ECOLOGICALLY-FRIENDLY PIG AND POULTRY PRODUCTION.

A WHOLE-SYSTEMS APPROACH TO OPTIMISING FEED EFFICIENCY AND REDUCING THE ECOLOGICAL FOOTPRINT OF MONOGASTRICS.

BASIC DATA

Funding:
EU-FP7
(€ 6 million)

Start date:
1 February 2013

Duration:
48 months
(2013 to 2016)
Assessing differences in small intestinal function in pigs of low and high residual feed intake

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Introduction

Gastro-intestinal tract (GIT) and liver
• important energy sinks
• need disproportionate amounts of energy relative to their weight

Contribute to diverging residual feed intake (RFI) in pigs

Low versus high RFI pigs:
Controversial results for differences in small intestine
• digestive & absorptive function
• morphology
• barrier function
• mucosal immune response
Hypothesis

- Low RFI is linked to enhanced digestive and absorptive capacity in pigs.
- Low RFI is associated with enhanced intestinal barrier function and reduced expression of pathogen-recognition receptors and cytokines.

Objective

to investigate differences in size, structure and function...
- intestinal morphology
- duodenal disaccharidase activity
- jejunal permeability
- gene expression in relation to sugar and short-chain fatty acid transport, tight-junction proteins and innate immune response

... in finishing pigs of diverging RFI.
Pig trial and sample collection

- 6 litters (♂ and ♀), n = 12 per pen
- Weighing once a week
- Transponder feeding from day 42 postweaning
- Wheat-barley-soybean meal based diet
- Selection of pigs using feed intake and ADG from day 42 to 98 postweaning
  - 8 low RFI pigs and 8 high RFI pigs (4 ♂ and 4 ♀ each)
- Gut tissue sampling (postprandially): days 102-105 postweaning

<table>
<thead>
<tr>
<th>Gut tissue</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Caeca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length + weight</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Histo-morphology</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Mucosal disaccharidases activity</td>
<td>✓</td>
<td></td>
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<tr>
<td>Ussing chamber experiment</td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Candidate gene expression</td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>
Lab analyses

**Ussing chamber experiment:**

Electrophysiological measurements:
- Continuous recording short-circuit current (Isc, µA/cm²), and
- Tissue resistance ($R_t$, Ω x cm²) -> tissue conductance ($G_t$, mS/cm²) ≈ 1 / $R_t$

Permeability marker measurements:
- Mucosal-to-serosal flux of fluorescein 5(6)-isothiocyanate (FITC) & horseradish peroxidase (HRP)

**Histo-morphology:**
- Formol-fixation and paraffin embedding
- Hematoxylin and eosin staining
- Villus height & width, crypt depth, goblet cells, intraepithelial lymphocytes

**Mucosal disaccharidases activity:**
- maltase (EC 3.2.1.20), sucrase (EC 3.2.1.48) and lactase (EC 3.2.1.23) activities in one gram protein (modified to Dahlquist, 1964)
- Released glucose was determined using glucose oxidase-peroxidase method
- Protein determination using Coomassie Blue dye-binding protein quantitation assay

**Candidate gene expression:**
- RNA isolation using bead-beating and RNeasy Midi Kit (Qiagen)
- Reverse-transcription-quantitative PCR using EvaGreen chemistry

**Statistical analysis:**
- ANOVA using PROC MIXED (SAS)
- least-squares means ± pooled SEM; significance: $P \leq 0.05$; trends: $0.05 < P \leq 0.10$
Residual feed intake, feed intake and growth of pigs of diverging feed efficiency

RFI, $P < 0.001$

Approx. 2 kg difference

Low RFI

High RFI

$P = 0.08$

kg/day

ADFI

ADG
Histo-morphology in pigs of low and high RFI

Questions that arise:
- Effects related to feed intake?
- Effects related to microbiota?
Low RFI in pigs:
- Does the greater lactase activity have relevance for RFI gain?
Ussing chamber results of low and high RFI pigs

Distal jejunum (2.5 m cranial to ileo-cecal junction)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low RFI</th>
<th>High RFI</th>
<th>SEM</th>
<th>RFI, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-circuit current (mA/cm²)</td>
<td>54.9</td>
<td>55.7</td>
<td>13.77</td>
<td>0.97</td>
</tr>
<tr>
<td>Tissue conductance (mS/cm²)</td>
<td>16.9</td>
<td>17.5</td>
<td>1.90</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**FITC = Fluorescein isothiocyanate; small molecular weight marker**

**HRP = horse-radish peroxidase; large molecular weight marker**

**RFI, P = 0.059**

**RFI, P = 0.017**

Low RFI in pigs: permeability ↑ in distal jejunum
Expression of target genes at jejunal mucosa in pigs of low and high RFI pigs

Distal jejunum (2.5 m cranial to ileo-cecal junction)

Candidate genes:

- **IL1B**: interleukin 1β
- **TNFA**: tumor necrosis factor-α
- **TLR2**: Toll-like receptor 2
- **TLR4**: Toll-like receptor 4
- **ALP**: intestinal alkaline phosphatase
- **MUC2**: mucin 2
- **MCT1**: monocarboxylate transporter 1
- **SGLT1**: sodium/glucose-co-transporter 1
- **OCLN**: occludin
- **ZO1**: zona occludens 1

Differences in mucosal response to luminal lipopolysaccharides in distal jejunum

Fold changes were calculated using the 2^{-ΔΔCq} method

*RFI, P < 0.1
**RFI, P < 0.05
Expression of target genes at jejunal mucosa in pigs of low and high RFI pigs

**Candidate genes:**

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Distal jejunum (2.5 m cranial to ileo-cecal junction)

Fold changes were calculated using the $2^{-\Delta\Delta Cq}$ method

No RFI effect
Summary and Conclusion

Low vs. high RFI:
- Differences in intestinal **structure** and **function**
- Jejunal gene expression ≠ observations at functional protein level

→ RFI gain was related to differences in innate immune response including jejunal barrier function
→ Effect of the **intestinal microbiota**
→ Effect of host-nutrient absorption
→ To clarify: effect of feed intake level and intestinal nutrient flow
Acknowledgments

- Vetmeduni
  - Lab staff of Inst. of Animal Nutrition and Functional Plant Compounds
  - PD Dr. Kirsti Witter (Inst. of Anatomy, Histology and Embryology)
- Prof. Dr. Jörg Aschenbach (Freie Universität Berlin, Inst. of Veterinary Physiology)
- Prof. Dr. Jürgen Zentek (Freie Universität Berlin, Inst. of Animal Nutrition)

Thank you!
Determination of feed efficiency

Selection of high and low feed efficient animals - based on Residual Feed Intake

Residual Feed Intake (RFI) = difference between observed and predicted feed intake, with lower RFI values indicating greater energy efficiency

Other measures of feed efficiency
  Feed conversion ratio
  Residual body weight gain
  Residual feed intake and body weight gain
Ussing chamber experiment

• The tissue was alternatively pulsed with a positive or negative pulse of 20 µA and 100 ms duration.
• The displacement in PD caused by the current pulse was measured, and the transepithelial tissue resistance \( R_T \) was calculated from the change in PD using the law of Ohm.
• After the equilibration period, the tissue was short-circuited by clamping the voltage to zero.

• Transepithelial potential difference \( (dP_0, \text{mV}) \), \( I_{sc} \) (µA/cm²) and \( R_T \) (Ω x cm²) were continuously recorded using a microprocessor-based voltage-clamp device and software (version 9.10; Mussler, Microclamp, Aachen, Germany).
• The magnitude of the clamp current is determined from PD and the series resistance of the circuit plus mucosa, and it was applied continuously by the automatic voltage clamp

\[
I_{sc} = \text{net sum of electrogenic charge transfer by the epithelium, which has the same magnitude but opposite direction to the externally applied clamp current.}
\]
### Ussing chamber results of low and high RFI pigs

#### Distal jejunum

#### Glucose response

<table>
<thead>
<tr>
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<th>High RFI</th>
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<th>RFI, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal short-circuit current (mA/cm²)</td>
<td>55.8</td>
<td>61.7</td>
<td>10.72</td>
<td>0.71</td>
</tr>
<tr>
<td>Δ short-circuit current (mA/cm²)</td>
<td>0.39</td>
<td>1.40</td>
<td>0.32</td>
<td>0.05</td>
</tr>
<tr>
<td>Basal tissue conductance (mS/cm²)</td>
<td>15.8</td>
<td>16.2</td>
<td>2.01</td>
<td>0.90</td>
</tr>
<tr>
<td>Δ tissue conductance (mS/cm²)</td>
<td>0.89</td>
<td>0.83</td>
<td>0.10</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Basal measurements were performed 1 min before glucose addition (concentration at mucosal chamber side: 10 mmol/L)

Δ short-circuit current and Δ tissue conductance = max. values obtained from 2 min after glucose addition minus basal values 1 min before glucose addition.