Faecal biomarkers for intestinal health in nutritional studies

Theo Niewold
Introduction

• Gut is crucial for health and growth

• In particular in high production animals

• Nutrients, barrier

• Immune system central in regulation
Immunity means costs

- If none: growth to 100% of genetic potential
- Main factor inflammation: reduction of growth
- Inhibition inflammation: back towards 100%
Immune systems

- Systemic (ca 30%)
  - reactive

- Mucosal (ca 70%)
  - tolerant (feed is foreign)
  - tight regulation
  - enhancement may cause pathology
Immune systems

• In both Systemic and Mucosal
  – innate (inflammation) and acquired (antibodies)
  – in both central role for macrophage

• Costs for growth:
  – antibodies up to 3%
  – inflammation 10-30%
• Inflammation causes
  – Lower appetite
  – Catabolism muscle
  – Disease/Pathology
  – Pathogens (eg. Campy)
  – Abdominal fat
INTESTINAL INFLAMMATION

• Is reciprocal to growth and health

• So (small intestinal) inflammatory biomarkers are promising
How to determine intestinal health

- Problems inaccessability GI-tract
  - necropsy
  - biopsy
  - fistulation
  - endoscopy

- All very invasive and expensive, alternatives?
Biomarkers

• Post-mortem: protein, mRNA expression in mucosa

• Less invasive: plasma acute phase proteins

• Non-invasive: faecal, urine, saliva

Alternatives 1

• Added markers: dual sugar methods e.g. lactulose/mannitol tests (urine/plasma)
  • testing permeability, but too variable
  • useless
Alternatives 2

- Spontaneous markers preferably
  - plasma
  - saliva
  - urine
  - faeces

Requirements:
- Less/non-invasive
- Reagents available
- Cheap
Important factors in intestinal function

- Integrity/permeability
- Other: inflammation, damage/infection

Common factor: Inflammation

Many available in human
<table>
<thead>
<tr>
<th>Protein/Marker</th>
<th>Location</th>
<th>Species</th>
<th>Body Fluid</th>
<th>Immunoassay Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal fatty acid binding protein</td>
<td>Intestine enterocyte damage</td>
<td>Porcine</td>
<td>Blood</td>
<td>Imm: Porcine, Chicken</td>
</tr>
<tr>
<td>Claudin 3</td>
<td>Tight junction loss, intestine permeability</td>
<td></td>
<td>Urine</td>
<td>Imm: Porcine</td>
</tr>
<tr>
<td>Pancreatitits associated protein (PAP, Reg3)</td>
<td>Intestine inflammation</td>
<td>Porcine</td>
<td>Faeces</td>
<td>Imm: Porcine</td>
</tr>
<tr>
<td>Citrulline</td>
<td>Small intestine epithelial loss</td>
<td>Absent in chicken</td>
<td>Blood</td>
<td>Imm: Porcine</td>
</tr>
<tr>
<td>Inflammatory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloperoxidase (MPO)</td>
<td>Intestine inflammation</td>
<td>Absent in chicken</td>
<td>Faeces</td>
<td>Imm: Porcine, Biochem: Porcine</td>
</tr>
<tr>
<td>S100 calmodulin</td>
<td>Intestine inflammation</td>
<td></td>
<td>Faeces</td>
<td>Imm: Porcine</td>
</tr>
<tr>
<td>Calprotectin</td>
<td>Intestine inflammation</td>
<td></td>
<td>Faeces</td>
<td>Imm: Porcine</td>
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<tr>
<td>Lactoferrin</td>
<td>Intestine inflammation</td>
<td></td>
<td>Faeces</td>
<td>Imm: Porcine</td>
</tr>
<tr>
<td>HMGB1</td>
<td>Intestine inflammation</td>
<td></td>
<td>Faeces</td>
<td>Imm: Porcine, Chicken</td>
</tr>
<tr>
<td>Lipocalin 2</td>
<td>Intestine inflammation</td>
<td></td>
<td>Faeces</td>
<td>Imm: Porcine</td>
</tr>
<tr>
<td>Neopterin</td>
<td>Intestine inflammation</td>
<td></td>
<td>Faeces</td>
<td>Imm: Porcine, Chicken</td>
</tr>
<tr>
<td>Acute phase proteins (haptoglobin)</td>
<td>Intestine inflammation</td>
<td>Porcine</td>
<td>Blood</td>
<td>Imm: Porcine, saliva</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saliva</td>
<td>Biochem: All</td>
</tr>
</tbody>
</table>
### A. Growth and serum acute phase proteins

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control pigs (n=13)</th>
<th>OTC pigs (n=14)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Weight gain (kg, 37d)</td>
<td>8.5</td>
<td>2.4</td>
<td>10.4</td>
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<tr>
<td>Haptoglobin (mg/mL)</td>
<td>0.78</td>
<td>0.60</td>
<td>0.45</td>
</tr>
<tr>
<td>SAA (mg/mL)</td>
<td>101.0</td>
<td>46.6</td>
<td>71.8</td>
</tr>
</tbody>
</table>

NB: APP are also influenced by other inflammatory processes
Pig Intestinal: analogous to mice/man

• Enterocyte (Small Intestine) markers:
  - Intestinal Fatty Acid Binding Protein (IFABP): cell damage
  - Pancreatitis Associated Protein (PAP/Reg3): inflammation
  - Claudin 3: permeability (link inflammation)

• Inflammatory cell markers:
  - Myeloperoxidase (MPO (inflammation), in faeces
  - many more (also from inflammatory bowel disease)
IFABP pig

- Marker for acute enterocyte damage
- Human ELISA cross-reacts
- Plasma, urine, faeces

Results post weaning piglets
Enterotoxigenic *E. coli* test post infection

**I-FABP faeces Rec status**

<table>
<thead>
<tr>
<th>Days</th>
<th>5+</th>
<th>5-</th>
<th>13+</th>
<th>13-</th>
<th>19+</th>
<th>19-</th>
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</thead>
<tbody>
<tr>
<td>Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pg/mg wet weight</td>
<td>7500</td>
<td>5000</td>
<td>2500</td>
<td>1000</td>
<td>500</td>
<td>250</td>
</tr>
</tbody>
</table>
IFABP results and to do

• Biomarker for *acute* enterocyte damage in pigs

• In plasma, urine and faeces

• In pigs,
  – Chicken similar protein?
MPO Faeces pigs (3 additives)

Haptoglobin (Hp) measure in plasma is reciprocal to growth (standard)

MPO in faeces correlates with Hp

MPO can be simply measured by colorimetric assay (peroxidase)

Cheap and no specific antibodies required

Successful additives show 50% reduction in faecal MPO
Study milk replacers

MPO parallels growth (retardation)

(MPO not in chicken)
• Inflammatory marker
  – pancreatitis associated protein, also Reg 3
  – antibacterial, anti-inflammatory

• Correlates with severity of e.g. infection (ETEC)

• Described to be present in other species in plasma, urine, faeces
• Works at the mRNA level, not protein (ELISA) – despite claims from companies

• Problem appeared to be:

• Now specific pig antibodies, and testing (see next ppt) successfully
Concluding remarks 1

- Intestinal health and function in mammals can be determined by using faecal biomarkers.

- Still some validation has to be done.

- However, a good correlation is found between faecal biomarkers and growth.
Concluding remarks 2

• Inflammatory biomarkers such as PAP and MPO give similar results as in other species

• Faecal MPO is the simplest and cheapest

• Further field testing required

• End goal: animal side test
• Often parameters are used which not necessarily directly related to health and growth (villus/crypt ratio, microbiota etc)

• As opposed to inflammatory biomarkers (IB)

• IB for preventive and curative purposes

• Objective parameters for the efficacy of additives
Concluding remarks 4

- Particularly relevant because of search for alternatives to antimicrobial growth promoters (AGP) and Zn

- These are anti-inflammatory agents

- So alternatives should be too

- Prove by low MPO (or PAP etc)
Thank you

Questions?

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