The intestinal innate immune response, mechanisms and implications for feed composition and feed additives

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
• 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. Clostridium)
  – Intestinal permeability
INTESTINAL INFLAMMATION

- Causes: stress, metabolic inflammation etc
- Is reciprocal to growth and health
- Should be inhibited
Table 2. The relationship between the direct anti-inflammatory properties of antibiotics and their use as antimicrobial growth promoters (AGP). Adapted after Niewold, 2007.

<table>
<thead>
<tr>
<th>Type of antibiotic</th>
<th>Anti-inflammatory</th>
<th>use as AGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-lactams</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Cyclines</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Quinolones</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Macrolides</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Peptides (e.g. Zn-Bacitracin)</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>
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

• 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: metabolic inflammation, damage/infection, stress

Common factor: Inflammation

Many available in human
<table>
<thead>
<tr>
<th>Enterocytes</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Intestinal fatty acid binding protein (I-FABP)</td>
<td>small intestine</td>
<td>porcine</td>
<td>• blood Imm: porcine, chicken</td>
</tr>
<tr>
<td>• Claudin 3</td>
<td>tight junction loss, intestine permeability</td>
<td>porcine</td>
<td>• urine Imm: porcine, chicken</td>
</tr>
<tr>
<td>• Pancreatitis associated protein (PAP, Reg3)</td>
<td>small intestine inflammation</td>
<td>porcine</td>
<td>• faeces Imm: porcine</td>
</tr>
<tr>
<td>• Citrulline</td>
<td>small intestine epithelial loss</td>
<td>porcine, absent in chicken</td>
<td>• blood Imm: porcine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inflammatory</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Myeloperoxidase (MPO)</td>
<td>intestine inflammation</td>
<td>absent in chicken</td>
<td>• faeces Imm: porcine</td>
</tr>
<tr>
<td>• S100 calmodulin</td>
<td>intestine inflammation</td>
<td></td>
<td>• faeces Imm: porcine, chicken</td>
</tr>
<tr>
<td>• Calprotectin</td>
<td>intestine inflammation</td>
<td></td>
<td>• faeces Imm: porcine</td>
</tr>
<tr>
<td>• Lactoferrin</td>
<td>intestine inflammation</td>
<td></td>
<td>• faeces Imm: porcine</td>
</tr>
<tr>
<td>• HMGB1</td>
<td>intestine inflammation</td>
<td></td>
<td>• faeces Imm: porcine, chicken</td>
</tr>
<tr>
<td>• Lipocalin 2</td>
<td>intestine inflammation</td>
<td></td>
<td>• faeces Imm: porcine</td>
</tr>
<tr>
<td>• Neopterin</td>
<td>intestine inflammation</td>
<td></td>
<td>• faeces Imm: all Biochem: all</td>
</tr>
<tr>
<td>• Acute phase proteins (haptoglobin)</td>
<td>intestine inflammation</td>
<td>porcine</td>
<td>• blood Imm: porcine, saliva Biochem: all</td>
</tr>
</tbody>
</table>
In pigs: serum acute phase protein (APP)

<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>A. Growth and serum acute phase proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight gain (kg, 37d)</td>
<td>8.5</td>
<td>2.4</td>
<td>10.4</td>
</tr>
<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

Niewold et al., Res Vet Sci 77: 89-91, 2004
Results post weaning piglets
Enterotoxigenic *E. coli* test post infection

**I-FABP faeces Rec status**

![Graph showing I-FABP faeces Rec status](image)
IFABP results and to do

• Biomarker for *acute* enterocyte damage in pigs

• In plasma, urine and faeces

• In pigs, maybe too acute in serum, faeces too?
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
Example calves MPO

Study milk replacers

MPO parallels growth (retardation)
• 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
PAP in pig

• Works at the mRNA level, not protein (ELISA)
  – despite claims from companies

• Problem appeared to be:

• Now specific pig antibodies, and testing
## PAP in pig faecal extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>MPO (mU/ml)</th>
<th>PAP (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>79</td>
<td>130</td>
</tr>
<tr>
<td>2</td>
<td>350</td>
<td>507</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Faecal score</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>202</td>
</tr>
</tbody>
</table>
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 (pre-selection in vitro (e.g. butyrate))

- In vivo: prove by low MPO (or PAP etc)
Thank you

Questions?

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