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Faecal biomarkers for intestinal health in nutritional studies

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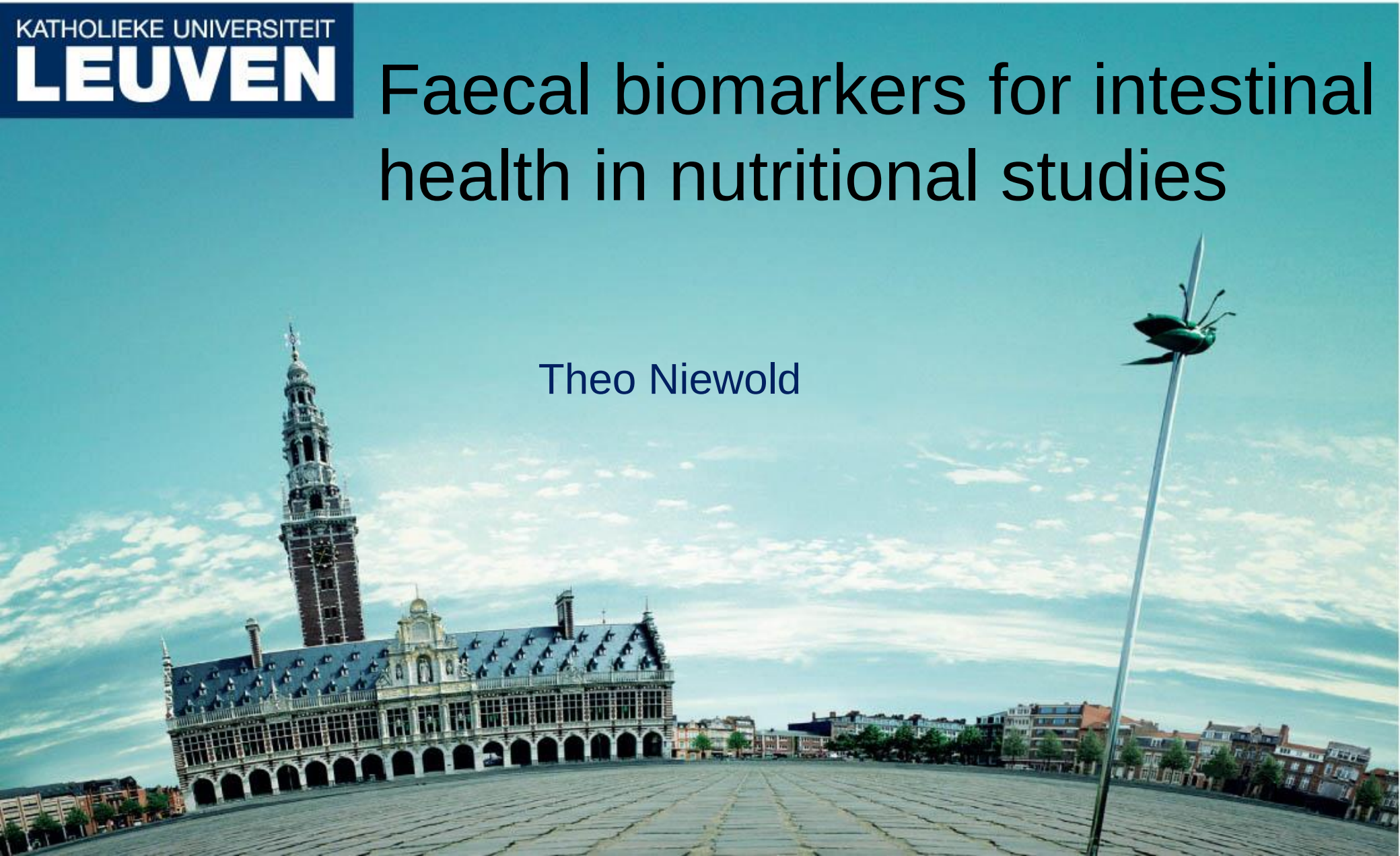


Introduction

- 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

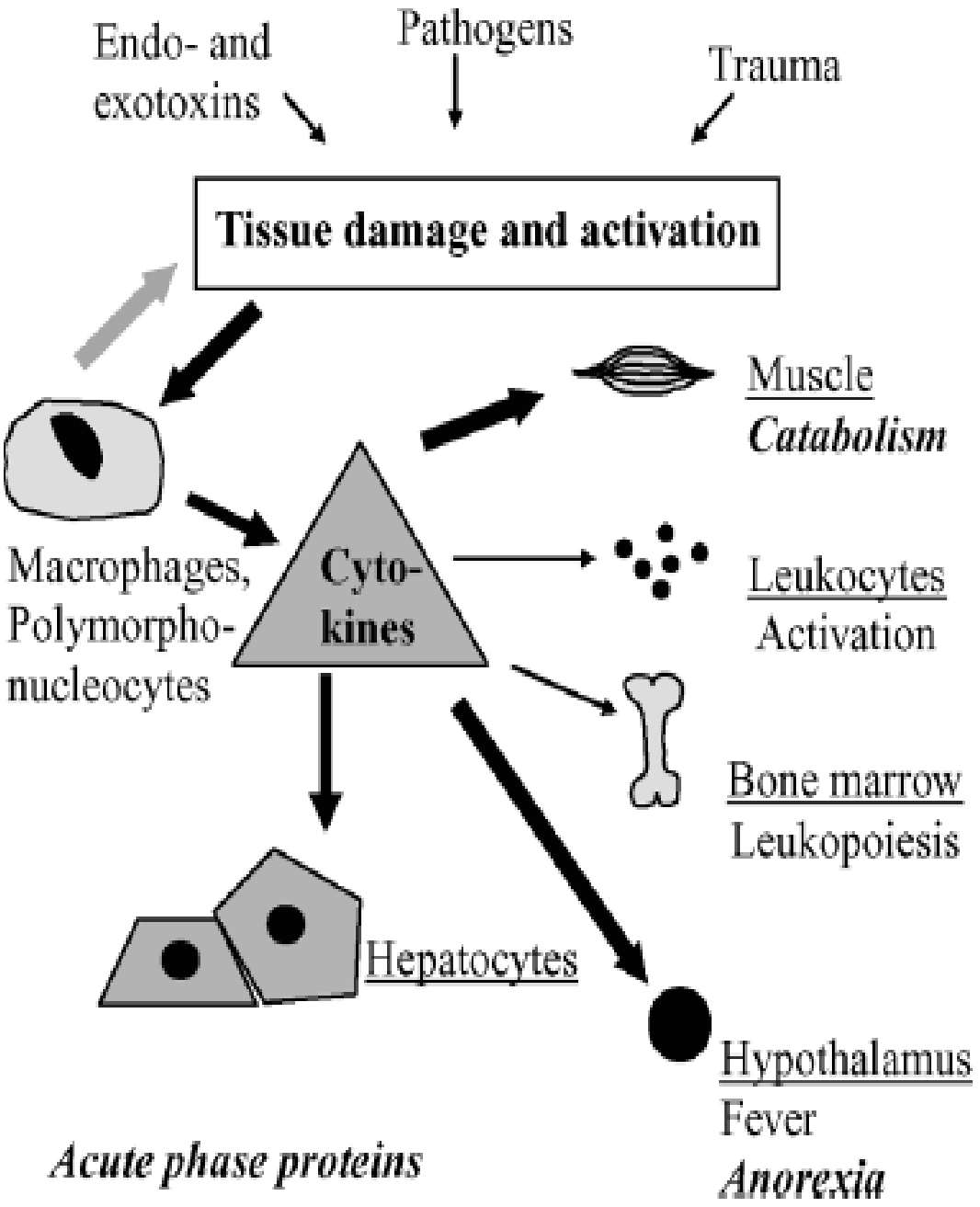
  - Mucosal (ca 70%)
    - tolerant (feed is foreign)
    - tight regulation
    - enhancement may cause pathology

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

Niewold TA. Intestinal health biomarkers in vivo. In: Intestinal health, key to maximise growth performance in livestock. Wageningen Academic Publishers, 2015 pp 219-228

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

Type?

Important factors in intestinal function

Integrity/permeability

Other: inflammation, damage/infection

Common factor: Inflammation

Many available in human

Enterocytes				
• Intestinal fatty acid binding protein (I-FABP)	small intestine enterocyte damage	porcine	• blood • urine • faeces[3]	Imm: porcine, *chicken*
• Claudin 3	tight junction loss, intestine permeability		• blood	Imm: porcine, *chicken*
• Pancreatitis associated protein (PAP, Reg3)	small intestine inflammation	porcine	• urine • faeces	Imm: *porcine*
• Citrulline	small intestine epithelial loss	porcine, absent in chicken	• blood	Imm: porcine
Inflammatory				
• Myeloperoxidase (MPO)	intestine inflammation	absent in chicken	• faeces	Imm: / Biochem: porcine
• S100 calmodulin	intestine inflammation		• faeces	Imm: *porcine, chicken*
• Calprotectin	intestine inflammation		• faeces	Imm: *porcine*
• Lactoferrin	intestine inflammation		• faeces	Imm: *porcine*
• HMGB1	intestine inflammation		• faeces	Imm: *porcine, chicken*
• Lipocalin 2	intestine inflammation		• faeces	Imm: *porcine*
• Neopterin	intestine inflammation		• faeces	Imm: all Biochem: all
• Acute phase proteins (haptoglobin)	inflammation	porcine	• blood • saliva	Imm: *porcine*, Biochem: all

In pigs: serum acute phase protein (APP)

Parameter	Control pigs (n=13)		OTC pigs (n=14)		*P*-value
	Mean	SD	Mean	SD	
A. Growth and serum acute phase proteins					
Weight gain (kg, 37d)	8.5	2.4	10.4	2.0	0.006
Haptoglobin (mg/mL)	0.78	0.60	0.45	0.30	0.107
SAA (mg/mL)	101.0	46.6	71.8	54.2	0.014

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:

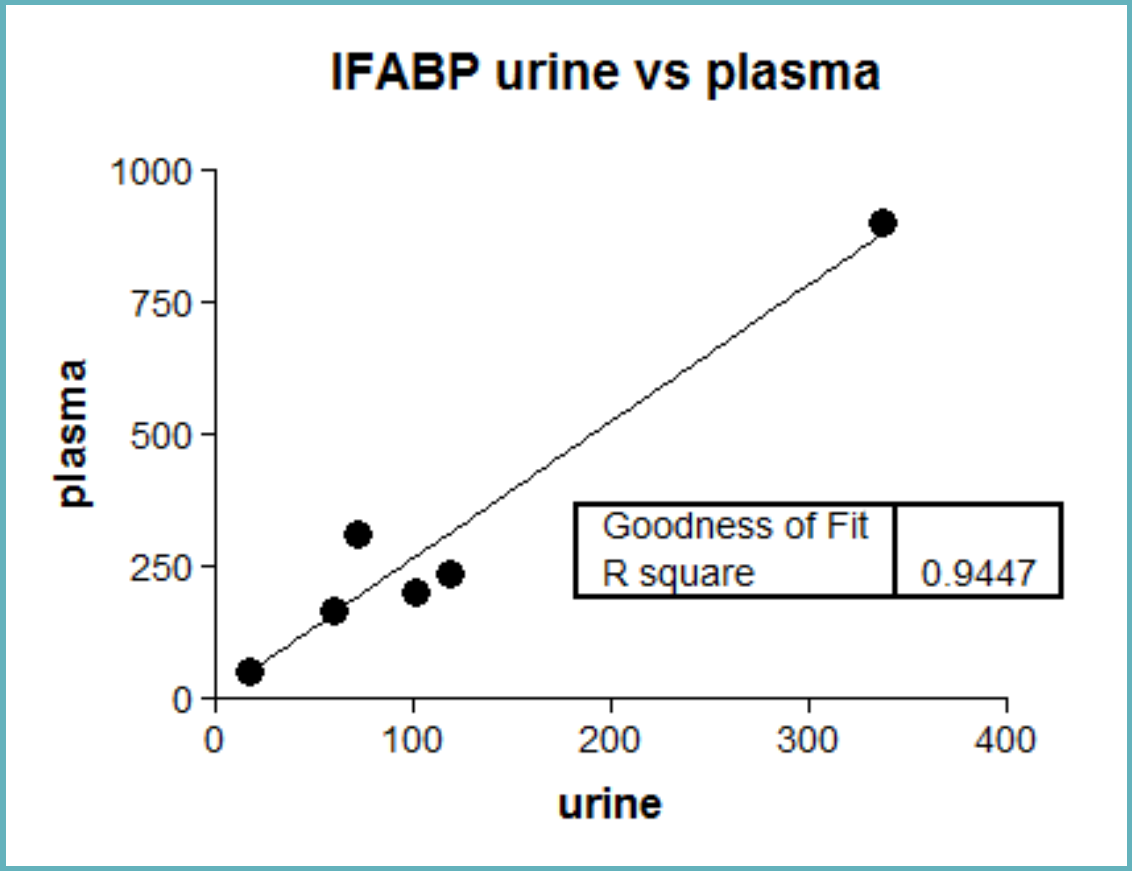
  -Myeloperoxidase (MPO (inflammation), in faeces
  -many more (also from inflammatory bowel disease)

IFABP pig

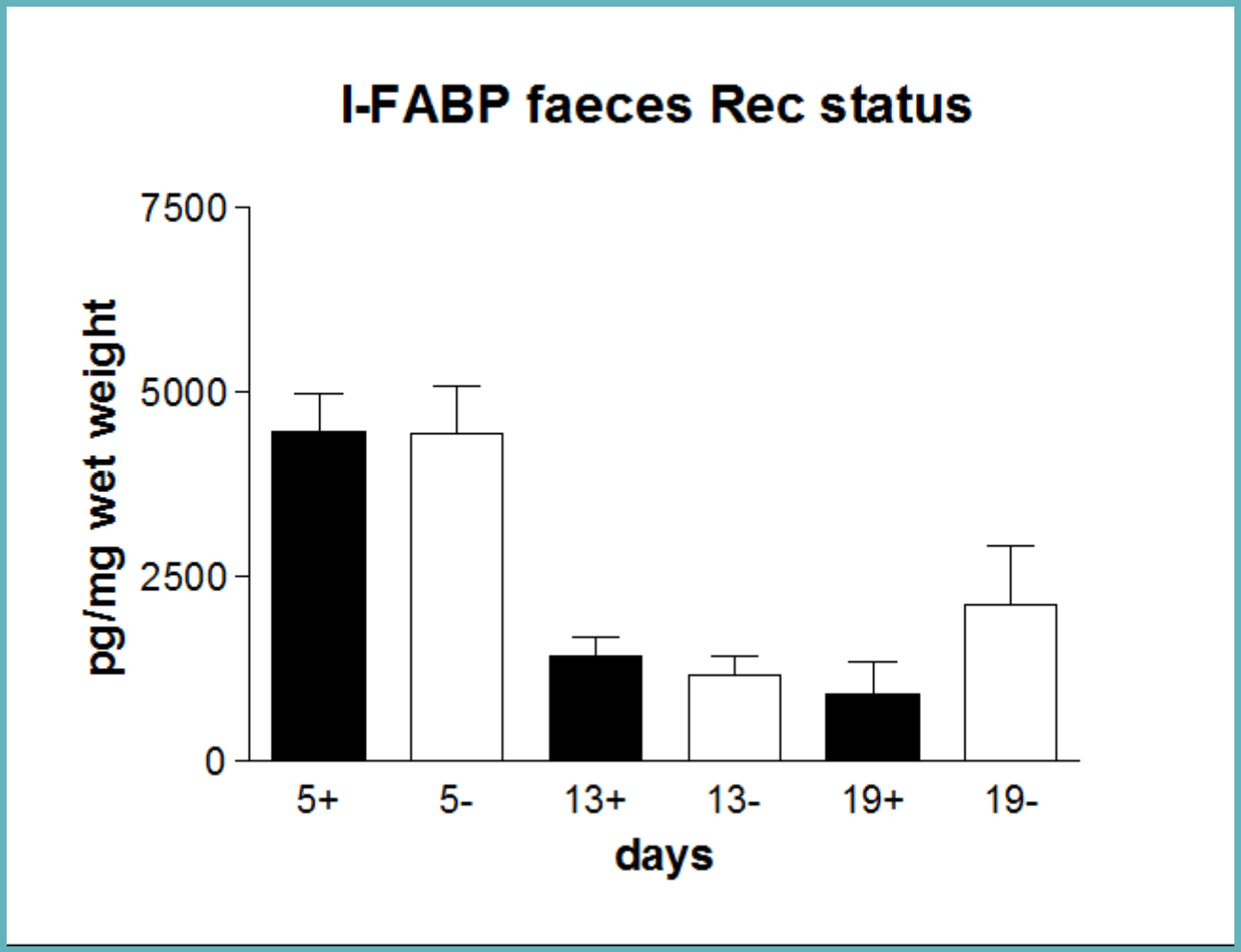
- Marker for acute enterocyte damage
- Human ELISA cross-reacts
- Plasma, urine, faeces

Niewold et al., *Res Vet Sci* 77: 89-91, 2004

Results post weaning piglets

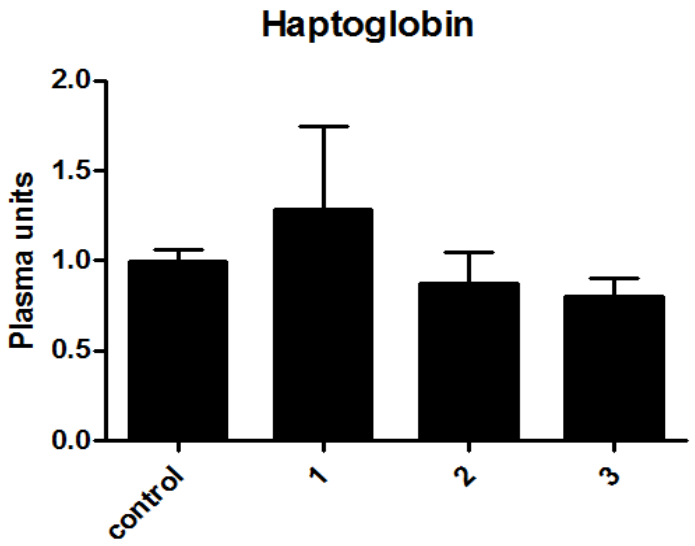


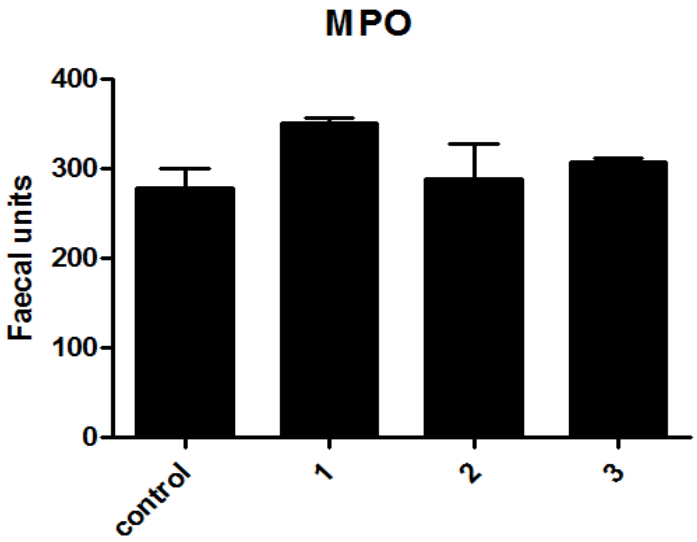
Enterotoxigenic *E. coli* test post infection



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

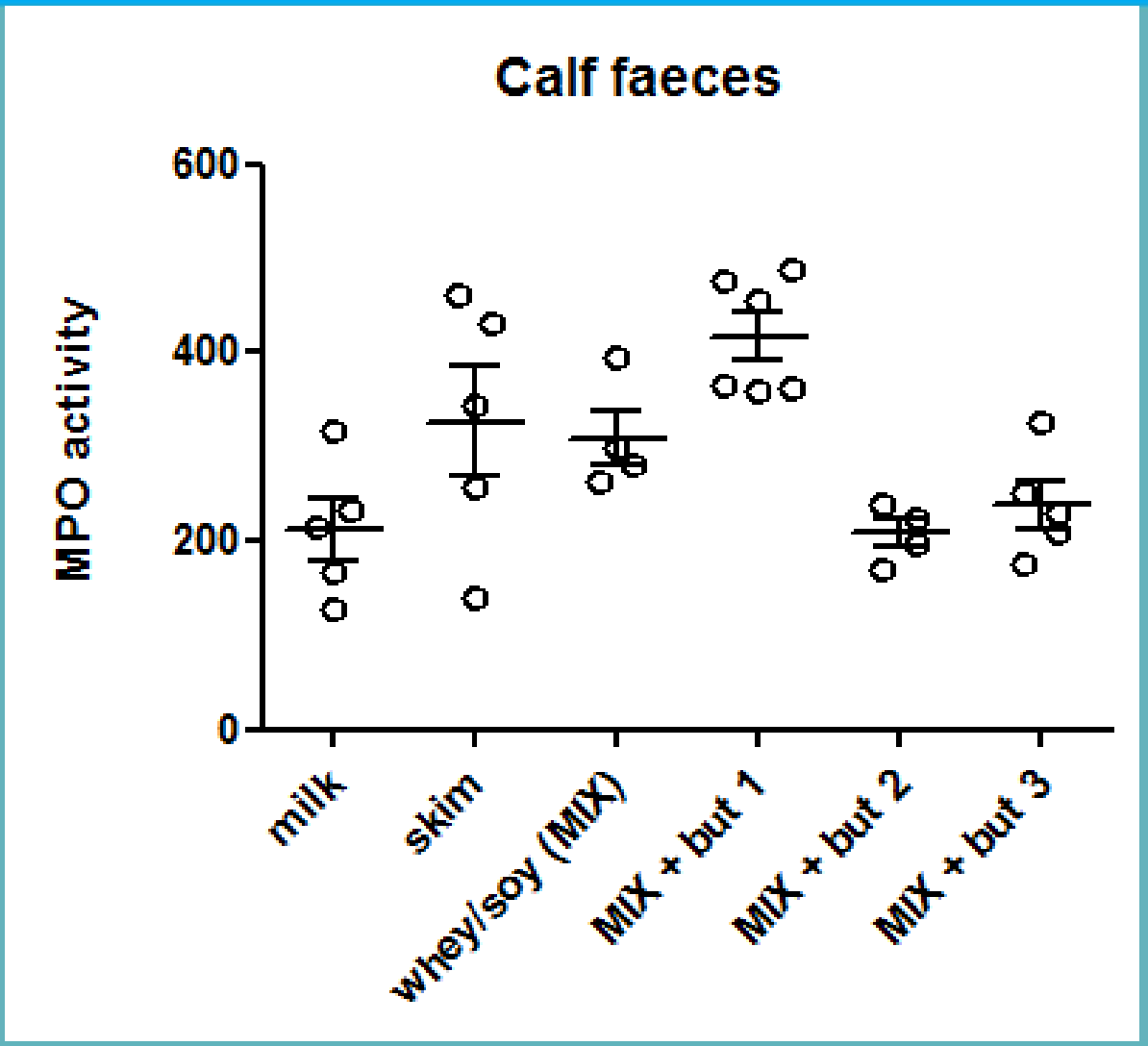
Successful additives show 50% reduction in faecal MPO

Example calves MPO

Study milk replacers

MPO parallels growth (retardation)

(MPO not in chicken)



PAP

- 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:
- Soler et al.:Identification of the major regenerative III protein (RegIII) in the porcine intestinal mucosa as RegIIIγ, not RegIIIα. *Vet Immunol Immunopathol*. 167:51–56, 2015
- 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

Concluding remarks 3

- 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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