Faecal Pig DNA: a potential non-invasive marker of gut cell loss

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Introduction

- Cell turnover

- Gut cell turnover rate is very high – high energy utilisation
- Currently all methods to assess gut cell loss – invasive
- Xylanase impacts upon gut health and therefore may impact upon gut cell loss
- Overall aim: to develop a non-invasive method for assessing gut cell loss utilising faecal samples from a pig xylanase trial
Hypothesis

Objectives of study:
1. Optimise pig DNA detection from faeces
2. Investigate any effects of an exogenous xylanase inclusion diet on pig faecal DNA
Target genes

- Faeces – heterogeneous material
  - Contains host, bacterial and feed DNA

- Difficult to detect host DNA – small proportion

- High copy number genes to test
  - Actin – conserved region across α, β and γ isoforms
  - Mitochondrial genes – Cytochrome b (CYTB)

- Bacterial DNA detection – universal primers (Mieszkin et al., 2009)
  - Total bacterial DNA content
Animal Trial Design

Pig weaner trial – wean – 10 weeks of age

Pigs were individually housed and fed *ad libitum* a basic feed, differing only in the addition of xylanase in the treated group.
Methods

**DNA extraction from faeces**
- Phenol chloroform based method
- DNA concentration normalised based on spectrophotometry – 50ng of DNA used per PCR

**Semi-quantitative PCR (40 cycles) & Gel electrophoresis**
- Assessing which host target gene is most sensitively detected

**Quantitative PCR**
- Assessing which host target gene is most sensitively detected
- Assessing the effects of xylanase on pig faecal DNA content

**Statistical analysis**
- Genstat 16th Edition
- Two-way ANOVA (treatment x day of trial)
- Assessing the effects of xylanase & time on pig faecal DNA content
Actin vs. CYTB – detection limits

- Testing a 2-fold dilution series of pig faecal DNA with either actin or CYTB primers

- CYTB amplicon – consistently detected
- Actin amplicon – amplification was unsuccessful with ↓ amount of DNA in the PCR reaction
PCR results

Actin amplicon:
- successfully detected by semi-quantitative PCR
- unsuccessful qPCR – detection at >35 cycles
- Low concentration of actin gDNA present in the faeces

CYTB amplicon:
- successfully detected by qPCR
- typically detected at ~22 cycles
- CYTB DNA > actin DNA conc. in faeces

Bacterial 16S amplicon:
- successfully detected by qPCR
- typically detected at ~15 cycles
- More bacterial DNA than either host gene
Effects of xylanase – host DNA

- **Actin amplicon** – trend (P=0.084) for a ↓ quantity in faeces of the xylanase treated group. No interaction or effect of time.
- **CYTB amplicon**: significantly (P=0.039) less in the xylanase treated group, trend (P=0.087) for an effect of time. No interaction.
Effects of xylanase – bacterial

- **Bacterial 16S amplicon:** no effect (P>0.05) of treatment or time. No interaction.
Conclusion

- Pig DNA in faeces - non-invasive marker of gut cell loss?
- CYTB DNA present in pig faeces at a higher concentration than actin DNA
- CYTB is a ‘better’ gene target for the detection of host DNA in pig faeces
- Xylanase reduced quantities of pig DNA in faeces
- Xylanase may reduce gut cell losses

Thank you for listening 😊