

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

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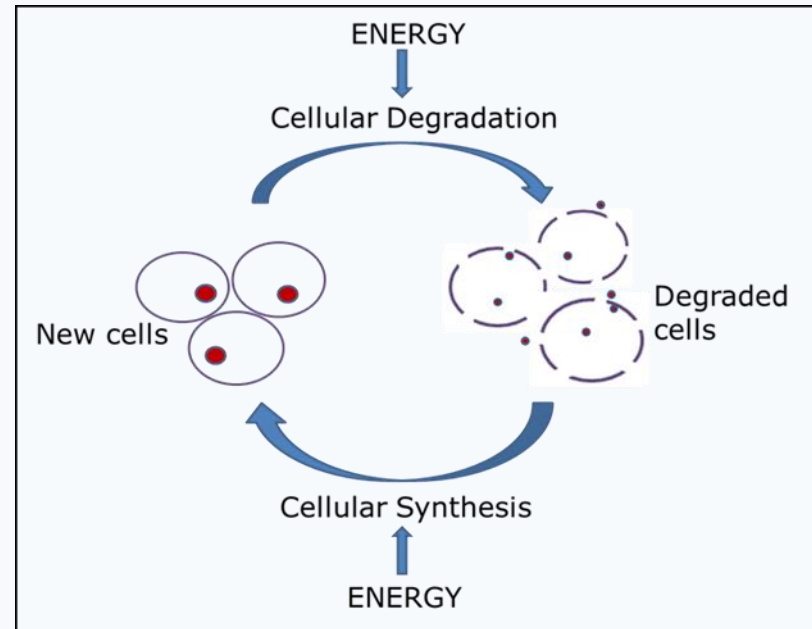
1 – University of Nottingham

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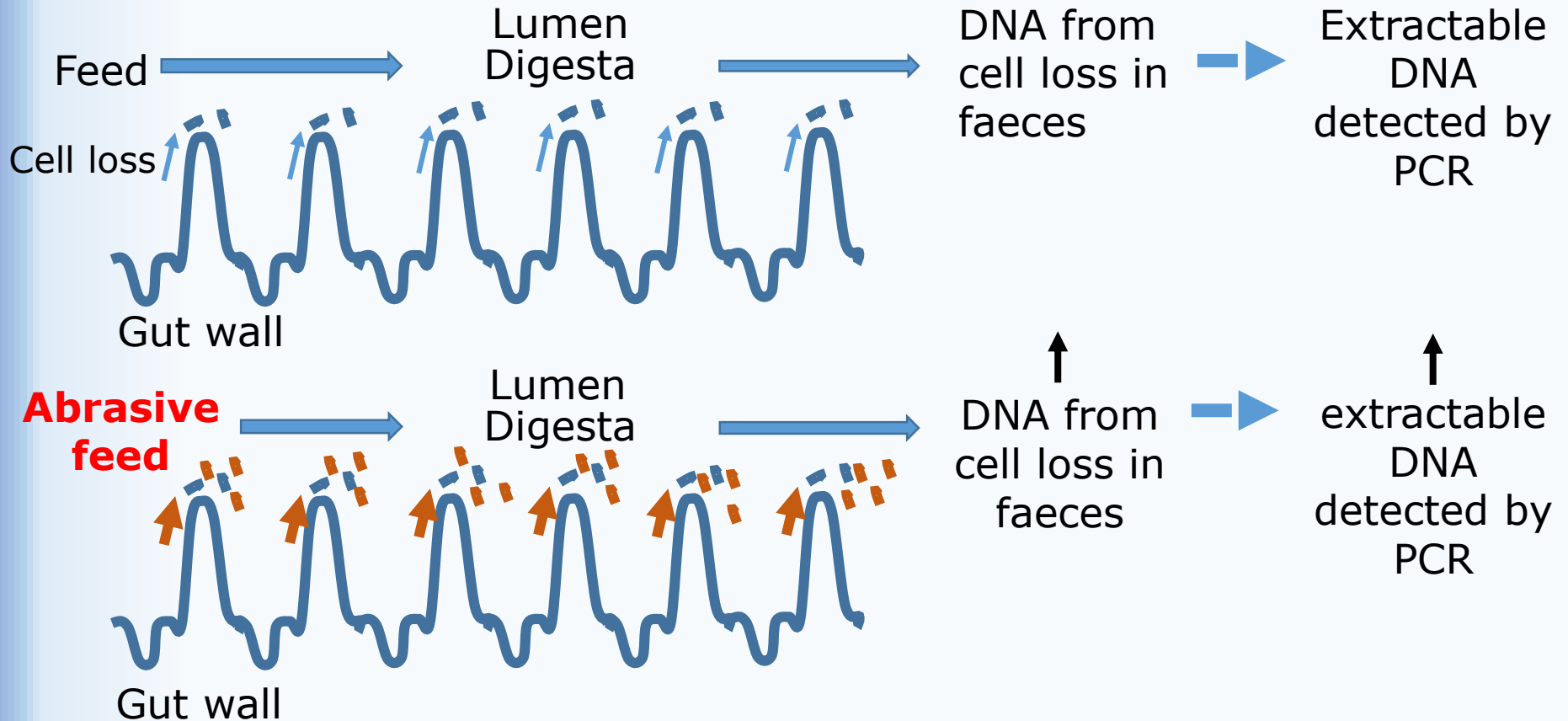
Introduction

- 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:

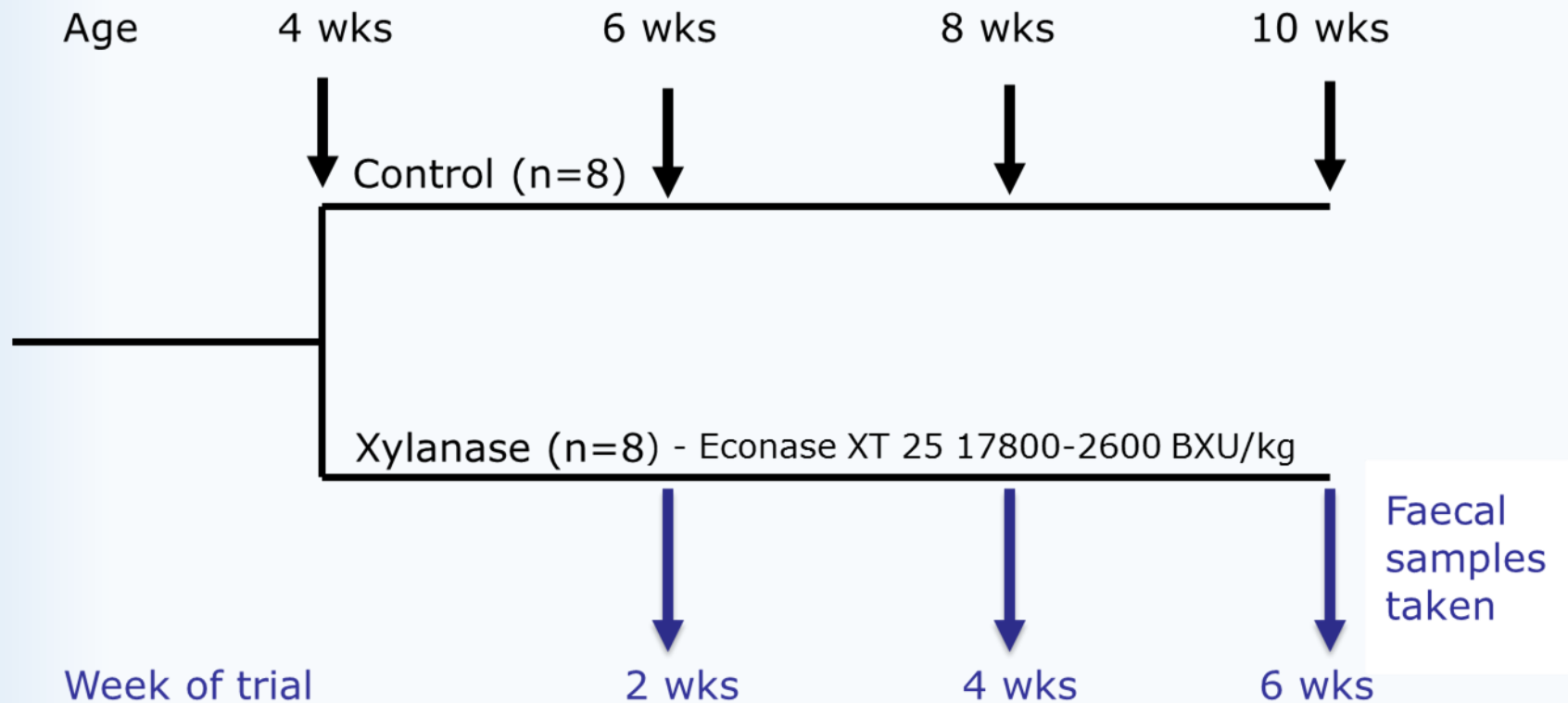
1. Optimise pig DNA detection from faeces
2. Investigate any effects of an exogenous xylanase inclusion diet on pig faecal DNA

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

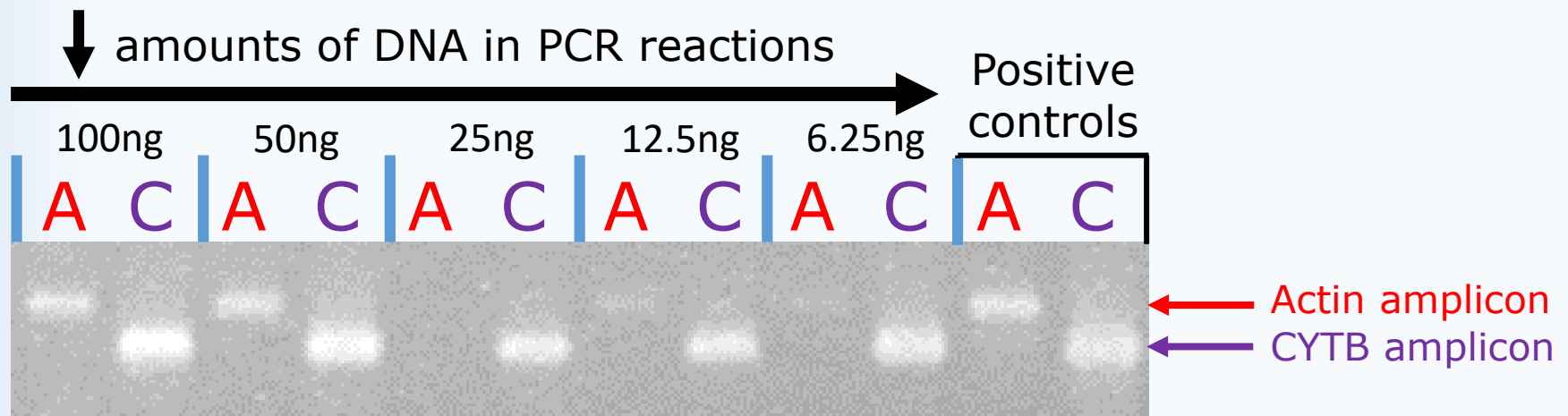
- Assessing which host target gene is most sensitively detected
- Assessing the effects of xylanase on pig faecal DNA content

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:

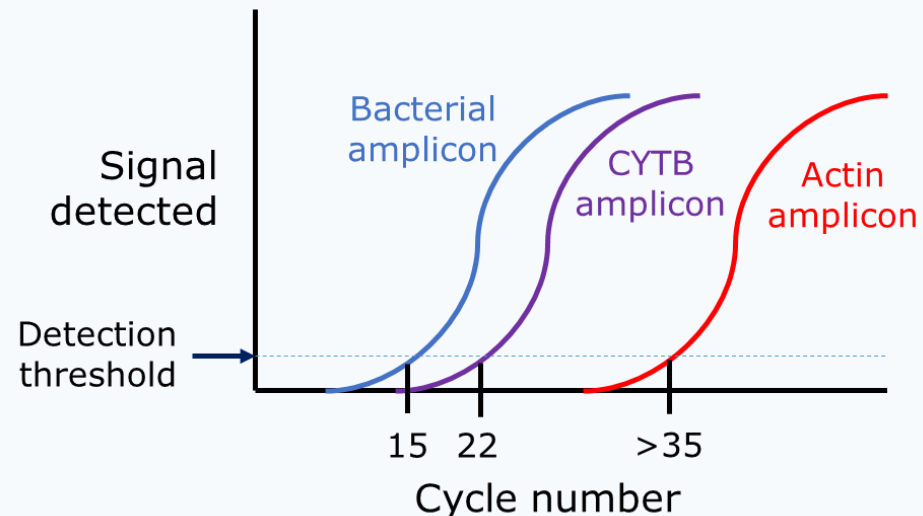
- successfully detected by semi-quantitative PCR
- unsuccessful qPCR – detection at >35 cycles
- Low concentration of actin gDNA present in the faeces

CYTB amplicon:

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

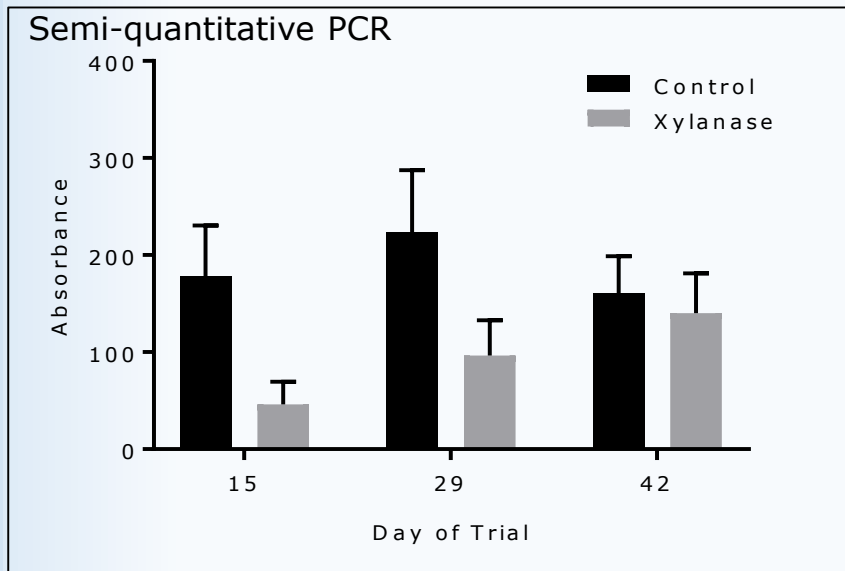
Bacterial 16S amplicon:

- successfully detected by qPCR
- typically detected at ~15 cycles
- More bacterial DNA than either host gene

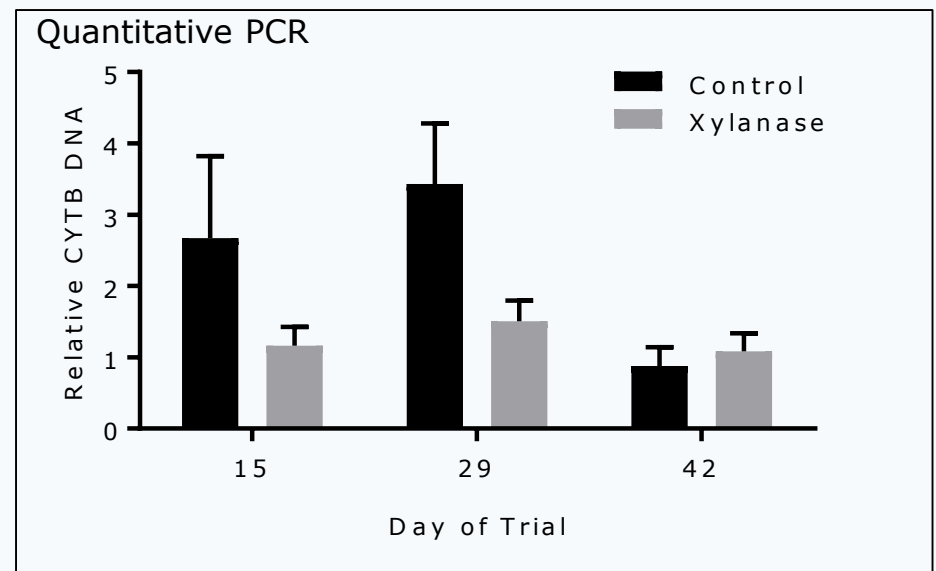


Effects of xylanase – host DNA

Actin DNA



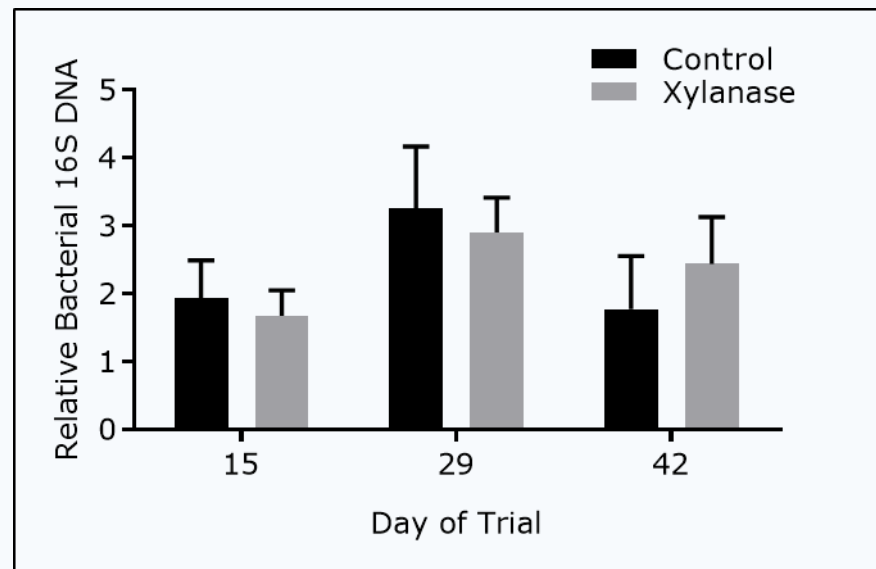
CYTB DNA



- **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 DNA



- **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 😊