Project Final Report

6 December 2013

Project Name:	Ratios of commensal to pathogenic bacteria as markers of pig intestinal health: development and validation of qPCR profiling assays
The fund:	$10,000 (Undergraduate Honours Award); Project No. in UWS: 20711.82617
Funding Organisation:	Pork CRC
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Background

In 2013, Bethany Bowring was awarded $10,000 by Pork CRC for her Honours study to be carried out at Elizabeth Macarthur Agricultural Institute (EMAI), Department of Primary Industry, NSW, under supervision of Dr Alison Collins and Dr Ming Wu.

The aim of the project was to establish efficient molecular assays based on quantitative polymerase chain reaction (qPCR) technology for quantifying the faecal bacterial population and determining ratios of beneficial to detrimental bacteria. This project is significant for the evaluation of pig intestinal health which is highly relevant to overall animal welfare and economic viability within the pork industry.

Half of the fund was set to pay Bethany as a stipend for supporting her study, and the other half for project-related expenditure.

Final summary of the project

Firstly, it is very pleasing to report that Bethany has done extremely well in her Honours project. She has worked hard and established a number of qPCR assays for quantification of faecal bacteria; and a thesis of high standard has been prepared. Although the final mark from the thesis examination has not been released yet, it is no doubt to her supervisors that she will attain the first class Honours.

In summary of the project, PCR primers and TaqMan probes designed for the 16S ribosomal RNA gene or the 16S-23S intergenic spacer region were selected to be specific for either *Cl. perfringens*, Lactobacilli, *E. coli* or *Enterobacteriaceae*. The results demonstrated that the chosen primers and probe were specific to *Cl. perfringens*. The initial primers and probe for the quantification of Lactobacilli were non-specific and insensitive. The subsequent modification of the probe allowed for an increase in annealing temperature yet did not improve the specificity or sensitivity. Another set of primers and probe from the published literature were identified as an alternative for the quantification of Lactobacilli. However, this qPCR was excluded due to 100 % homology of the forward primer to other bacteria including *Clostridium* species and *Enterococcus* species. The optimal set of primers and probe, chosen according to the published sequences, was finally found to be suitable for qPCR

quantification of Lactobacilli in pig faeces. Two probes were designed in this study, and were found to be specific towards *E. coli* or *Enterobacteriaceae* respectively and suitable for use in qPCR. Strong and significant correlations were observed between microbial culture techniques and qPCR for the quantification of *E. coli* ($\rho = 0.863$, $p < 0.001$) and *Enterobacteriaceae* ($\rho = 0.912$, $p < 0.001$). No significant correlation was found between the quantification of Lactobacilli using microbial culture and qPCR ($\rho = 0.495$, $p = 0.072$). Numbers of *Cl. perfringens* were below the qPCR limit of detection for thirteen out of the fourteen samples enumerated using both microbial culture and qPCR. Moderate but statistically significant correlations were identified between microbial culture techniques and qPCR for the determination of ratios of Lactobacilli to *E. coli* ($\rho = 0.684$, $p = 0.007$) and ratios of Lactobacilli to *Enterobacteriaceae* ($\rho = 0.705$, $p = 0.005$).

The established qPCR assays were used to quantify numbers of *Cl. perfringens*, Lactobacilli, *E. coli* and *Enterobacteriaceae* within a mixture of scouring and healthy porcine faecal samples collected from 120 pigs within the age groups of 6 to14 days or 2 to 3 weeks post-weaning. No significant correlation was found between the percentage water content and the log numbers of *Cl. perfringens* relative to Lactobacilli ($\rho = 0.283$, $p = 0.191$). Weak but statistically significant correlations were found between an increase in water content and a reduction in *E. coli* numbers relative to *Enterobacteriaceae* ($\rho = 0.279$, $p = 0.002$) and Lactobacilli ($\rho = 0.379$, $p < 0.001$), along with a reduction in *Enterobacteriaceae* numbers relative to Lactobacilli ($\rho = 0.378$, $p < 0.001$). These findings are not in agreement with the existing concept that increased faecal water content positively correlates to increased numbers of pathogenic bacteria relative to commensal bacteria. Therefore, the results may be a reflection of the complexity of the intestinal microflora as *Cl. perfringens* and *E. coli* are not the sole causative agents of scouring in piglets. Additionally, the microbial culture techniques and qPCR assays used in this study targeted all *E. coli* strains rather than focusing on pathogenic strains. Future work in the development of a reliable indicator for pig intestinal health would enable further evaluation into risk factors associated with scouring and potentially improve both economic viability and animal welfare within the Australian and worldwide pig industry.

Finally, the supervisors and the student are grateful to Pork CRC for providing support to this project. The fund was used as per the guideline, that is, $5000 was paid to Bethany as a stipend, and the other $5000 was used in experiment-related cost.