Investigation and subsequent manipulation of the intestinal microbiota of pigs, with a view to optimising feed efficiency

By

Ursula Mary McCormack (B.Ag.Sc)

A thesis submitted for the degree of Doctor of Philosophy

Waterford Institute of Technology

Research Supervisors

Dr. Gillian E. Gardiner

Dr. Peadar G. Lawlor

Submitted to Waterford Institute of Technology September 2017

Declaration

No element of the work described in this thesis has been previously submitted for a degree at this or any other institution. The work in this thesis has been performed entirely by the author.

Signature: _____

Date: _____

Table of Contents

Table of Tables

Table of Figures

List of abbreviations

Abbreviation	Explanation
Ab	Antibiotic
ACTB	Beta-Actin
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
ALPi	Intestinal Alkaline Phosphatase
AT	Austria
B2M	Beta-2 Microglobulin
BF	Back Fat
BP	Base Pair
CFU	Colony Forming Unit
CO_2	Carbon Dioxide
CON	Control
CP	Crude Protein
DM	Dry Matter
DP	Degree Of Polymerisation
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
ENA	European Nucleotide Archive
EU	Endotoxin Units
EXP	Experiment
FBS	Fetal Bovine Serum
FCE	Feed Conversion Efficiency
FCR	Feed Conversion Ratio
FE	Feed Efficiency
FIRE	Feed Intake Recording Equipment
FITC	Fluorescein Isothiocyanate
FM	Fresh Matter
FMT	Faecal Microbiota Transplantation
G:F	Gain to Feed ratio
G_	Genus
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GC	Gas Chromatography
GIP	Glucose-Dependent Insulinotropic Peptide
GIT	Gastro-intestinal tract
GLP1	Glucagon-Like Peptide 1
GLUT2	Faciliated Glucose Transporter Member 2
Hb	Blood Haemoglobin
HBSS	Hank's Balanced Salt Solution
HPRA	The Irish Health Products Regulatory Authority
I	Ionomycin
I.S.	*Incertae Sedis*
IEL	Intra-Epithelial Lymphocytes
IL-4	Interleukin- 4
IL-6	Interleukin- 6

Abbreviation	Explanation
IL-8	Interleukin- 8
INU	Inulin
KEGG	Kyoto Encyclopedia of Genes and Genomes
LAL	Limulus Ameobcyte Lysate
LI	Large Intestine
LPL	Lamina Propria Lymphocytes
LPS	Lipopolysaccharide
MCT1	Monocarboxylate Transporter 1
MD	Muscle Depth
MRD	Maximum Recovery Diluent
NGS	Next Generation Sequencing
NH_3	Ammonia
NI	Northern Ireland
NR	Not reported
OCLN	Occludin Encoding Gene
OTU	Operational Taxonomic Unit
P	Phosphorous
P_	Phylum
PBS	Phosphate Buffered Saline
PCA	Principal Component Analysis
PCoA	Principal Co-Ordinate Analysis
PCR	Polymerase Chain Reaction
PED	Porcine Epidemic Diarrhoea
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction on Unobserved Species
PMA	Phorbol Myristate Acetate
PRRS	Porcine Reproductive and Respiratory Syndrome
PTS	Phosphotransferase System
PW	Post-Weaning
qPCR	Quantitative PCR
RDP	Ribosomal Database Project Classifier
RFI	Residual Feed Intake
RG	Residual Gain
RIG	Residual Intake and Body Weight Gain
ROI	Republic Of Ireland
S.E.M.	Standard Error of the Mean
SGLT1	Sodium-Dependent Glucose Transporter 1
SI	Small Intestine
SMCT	Sodium-Coupled Monocarboxylate Transporter
TCA	Trichloroacetic Acid
TLR	Toll Like Receptor
TNFα	Tumour Necrosis Factor A
Unc	Uncultured
VFA	Volatile Fatty Acid
WC	Wilkins Chalgren
ZO1	Zona Occludens 1

Investigation and subsequent manipulation of the intestinal microbiota of pigs, with a view to optimising feed efficiency

Ursula Mary McCormack

Abstract

The objectives of this thesis were (1) to explore a possible link between the intestinal microbiota and feed efficiency (FE) in pigs and (2) to investigate microbiota-modulating strategies to improve FE. Two studies were conducted in pigs ranked on divergence in residual feed intake (RFI; a metric for FE); one in Ireland only and one across three geographical locations. Microbial diversity, composition and potential functionality were assessed in faecal and digesta samples, using high-throughput 16S rRNA gene sequencing. In both studies, RFI-associated microbes were identified, albeit most at low relative abundance, with increases in bacterial taxa associated with improved metabolism and health found in low RFI (highly efficient) pigs. These bacterial taxa could potentially be exploited in the future as biomarkers for FE, targets for nutritional strategies, or probiotics, to improve FE. However, no FE-associated microbial taxon was common to all geographical locations, highlighting the influence of rearing environment on the intestinal microbiome. Manipulation of the microbiome with a view to improving FE was investigated in two studies. Firstly, faecal microbiota transplantation (FMT) was performed in pregnant sows and/or their offspring. Although RFI tended to be lower (better FE), slaughter weight was reduced by FMT, and offspring harboured more potentially pathogenic and fewer beneficial microbes. Secondly, inulin was fed to weaner pigs born to sows in the FMT study. No improvements in growth were observed, but RFI was lower in inulin-fed pigs from FMT sows, and decreases in potentially pathogenic microbes were observed. While these findings have negative implications for the use of FMT to improve FE in pigs, they demonstrate the considerable impact of early life intestinal microbiota on pig growth. In conclusion, the work from this thesis demonstrates a possible link between the intestinal microbiota and FE in pigs, but further work is needed to investigate causality.

1. Literature Review

1.1 Introduction

As feed costs account for ~70% of the total costs of producing a pig (Teagasc, 2016), feed efficiency (FE) is critical to the profitability of pig production. As a result, ways to improve FE in pigs are continually being sought. However this is by no means an easy task, as FE is affected by a number of factors, both animal-related (genetics/breed, gender, health), and husbandry-related (diet, farming system/rearing environment, management) (Li et al., 2016).

It is well known that the porcine intestinal microbiota plays a crucial role in utilising substrates otherwise indigestible to the host and is also involved in gut health and immunity (Fouhse et al., 2016, Mach et al., 2015, Willing, 2010). However, while in humans and rodents, there is a body of evidence demonstrating how the intestinal microbiota affects energy metabolism, this has not yet been fully established in pigs. Nonetheless, it is likely that there is a link between the intestinal microbiota and FE in pigs. Consequently, interventions targeting the intestinal microbiota could potentially improve FE and these may offer a number of advantages over traditional means of improving FE, e.g. genetic selection or re-design of farming systems which require extensive resources and are costly. Ways of manipulating the microbiota in order to improve FE include probiotics, prebiotics and other feed additives that aim to increase beneficial bacterial populations.

The aim of this literature review is to outline the factors that influence FE in pigs and to explore the potential contribution of the intestinal microbiota and intestinal microbiota-based strategies of improving FE in pigs.

1.2. Feed efficiency measures

There are a number of different measures which are traditionally used for FE. Table 1.1 outlines those most commonly used in all livestock animals; a lower number indicates a better, more feed efficient animal.

Table 1.1. Feed efficiency measures

Measure	Calculation	Description	Reference
Feed Conversion Efficiency (FCE)	Feed intake/ weight gain	The amount of feed taken to produce one kg of gain	(Bereskin, 1986)
Feed Conversion Ratio (FCR)	Feed intake (ADFI)/ weight gain (ADG)	The ratio of feed intake to product/kg gain (used interchangeably with FCE)	(McPhee, 1981)
Gain to Feed ratio (G:F)	Rate of feed conversion × feed + error (ADG/ADFI)	Indicates how much the animal actually needs to be fed	(Koch, 1963)

However, there are some limitations with these traditional FE measures, as these measures rely only on feed intake and growth rate, and so can be misleading, as a pig can have a low feed intake and a poor growth rate, but can have a 'good' FE as indicated by a low FCE/FCR value. Table 1.2 shows some more modern FE measures, which are considered more accurate.

Table 1.2. Newer feed efficiency measures

Term	Calculation	Description	Reference
Residual Feed Intake (RFI)	Observed intake- expected intake Back fat, muscle depth and gender included in the model also	Difference between actual feed intake and expected intake (lower number= more efficient)	(Herd, 2009, Savietto et al., 2014, Patience et al., 2015)
Residual Feed Intake and Body Weight Gain (RIG)	-1 × RFI and RG RIG= (RG/std. RG)-(RFI/std. RFI)	Residual gain (RG): difference between observed gain and that predicted by growth (higher number = more efficient)	(Berry et al., 2012)

Other less commonly used measures of FE are:

- Carcass feed conversion efficiency (FCE), which calculates the amount of feed per kg of carcass weight
- Energy FCE, which describes the amount of feed utilised for energy
- Lean FCE, which is the amount of feed converted to lean meat
- Edible FCR, which refers to producing more edible protein/energy than what is consumed

(Harris, 1970, Patience et al., 2015)

1.3. Factors affecting feed efficiency in pigs

There are numerous factors that determine how efficient an animal is in terms of growth and maintenance of baseline body requirements, but also in relation to producing a better quality carcass in order to meet consumer demands and market targets (Reese et al., 1985). Many of these factors are well established including genetics, diet composition and feed form, as well as management factors, and these will be discussed here.

1.3.1 Breed/genetics

Breed /genetics has a strong influence on FE in pigs. Numerous studies have been carried out to investigate the effect of breed on FE (Mrode, 1993, Saintilan et al., 2013). Each breed brings with it its own benefits and pitfalls; Duroc breeds will increase growth, but in order to increase the lean content of pig meat, the Large White (Yorkshire) should be included, and the Landrace can improve FCR (Mrode, 1993, Chen, 2002). Genetics can be manipulated to suit the needs of both producers and consumers of pig meat. Different breeding companies (e.g. Hermitage Genetics, PIC, DanBred) supply pig producers with semen from boars deemed optimal (based on FCE, lean meat %, reduced days to slaughter etc.) for artificial insemination (Hermitage, 2017, PIC, 2017, DanBred, 2017). This means that producers can make a decision based on the work performed by the breeding company so that they can produce the best possible offspring in terms of growth and carcass quality.

1.3.2. Diet

Diet is a major factor contributing to varying FE in pigs, and in theory it can be easily altered to optimise feed intake and growth. However, diet optimisation is a tedious process with uncertain results due to variability in composition, feed form, quality etc. (Just, 1984, Patience et al., 2015). These will be further discussed below.

1.3.2.1. Diet composition

Diet composition is a hugely important component of pig production, where the main nutrients are protein, carbohydrates and lipids. An improvement in the quality of ingredients, as well as changing the protein and fibre content, can improve average daily gain (ADG) and FE in weaner pigs (Wellock, 2009, Hermes, 2009), and the combination of a high fibre and low protein diet can improve feed intake and growth rate also (Bikker et al., 2006). The addition of fat to the diet can reduce feed intake, and improve ADG and FE (Collins, 2009). Diet formulation for pigs is an area that requires attention, due to the need to formulate based on least cost ingredients, the use of by-products, amino acid balance etc. However, the use of by-products/cheaper ingredients can have downfalls, including variability in composition, nutritional content, etc. Investigating the use of different nutrients and ingredients, and improving the quality and nutrient content of the diet in pig feed can affect growth performance and improve FE. Nutrient utilisation for growth and production are essential in achieving better FE.

1.3.2.2. Diet form

Diet form (particle size, pelleting, extrusion, cooking, liquid/dry, etc.) plays a significant role in the optimisation of nutrient absorption and maximisation of pig growth. Pelleting is a better choice for improving ADG and feed intake (Myers, 2013),

while reducing the particle size of feed by grinding improves FE (Choct, 2004). However, the incidence of stomach ulcers can increase in feed that is too finely ground (Millet, 2012). Processing (i.e. cooking) of dietary ingredients can improve FE, as it improves the availability of some nutrients (Wondra, 1995), but it can lead to burning and therefore a reduction in the nutritional quality of the diet. Extrusion (heat treatment) can be used to improve the nutritional content of dietary ingredients that may contain anti-nutritional factors such as peas. This may mean an improvement growth rate and FE, as increased nutrient utilisation from the diet and therefore a reduction in the amount of nutrients excreted may occur (Tuśnio et al., 2017). If alternatives to more expensive ingredients can be used, this is of further benefit to the producer, given the high cost of feeding pigs

Liquid feed is another form of feeding the diet and it is commonly used in Ireland, with ~70% of Irish pigs liquid-fed (Teagasc, 2015). It involves mixing water with the feed in a ratio of ~2:1. As liquid feed is more similar to the milk diet that newly weaned piglets are accustomed to, this can have huge benefits in terms of improving FE in weaners. Studies have shown that ADG and FCE are improved in grow-finisher pigs (Canibe et al., 2003, Hurst et al., 2008, Dung et al., 2005) but conflicting results have also been found, where liquid feeding reduced ADG (Lawlor et al., 2002). As liquid feeding results in microbial fermentation, good management practices are needed to ensure elimination of pathogenic bacteria that can build up in the mixing tanks and/or pipes used to transport feed (Missotten et al., 2015).

1.3.3. Feeding to match requirements

Feeding to match the requirements of pigs at particular growth stages can reduce the costs of production and potentially improve FE. Precision feeding is used to deliver

the required amount of feed, with the correct nutrient composition at the right time in their productive life, in order to improve FE, while reducing the excretion (and therefore nutrient loss) of nitrogen and phosphorous from the pig (Yang, 2008). Compensatory growth, where improved growth and FE are observed in pigs after a period of feed restriction, can be used to reduce the amount of feed given at all stages of the production cycle (Fabian, 2004). Matching the nutrient requirements of pigs to optimise FE can be a useful way to reduce feed costs as well as increasing nutrient utilisation from the feed (Pomar, 2014). These methods are quite useful as they can reduce the amount of feed, and therefore reduce the cost of production, as well as the pig potentially reaching slaughter weight faster. However, these approaches can involve a higher level of labour.

1.3.4. Management and microenvironment

Optimal management practices are critical in pig production. Producers need to maintain correct management practices, as well as controlling and monitoring everyday production in order to optimise efficiency and profitability. Management factors that can affect FE include temperature, ventilation/humidity, space allowance and grouping, light, and feeder type.

In nature, pigs display seasonal behaviours, but with domestication and intensification of productivity, requirements have changed. Temperature is one of these, for example; FE has improved but at the expense of dependency on an optimum microclimate. Pigs operate in a thermo-neutral zone (Kingma et al., 2012), where they require an optimum temperature to perform best, depending on age. As pigs age, their temperature requirements change, as shown in Figure 1.1; and if the temperature is too high or indeed too low, maintenance and growth will be affected, thereby affecting FE.

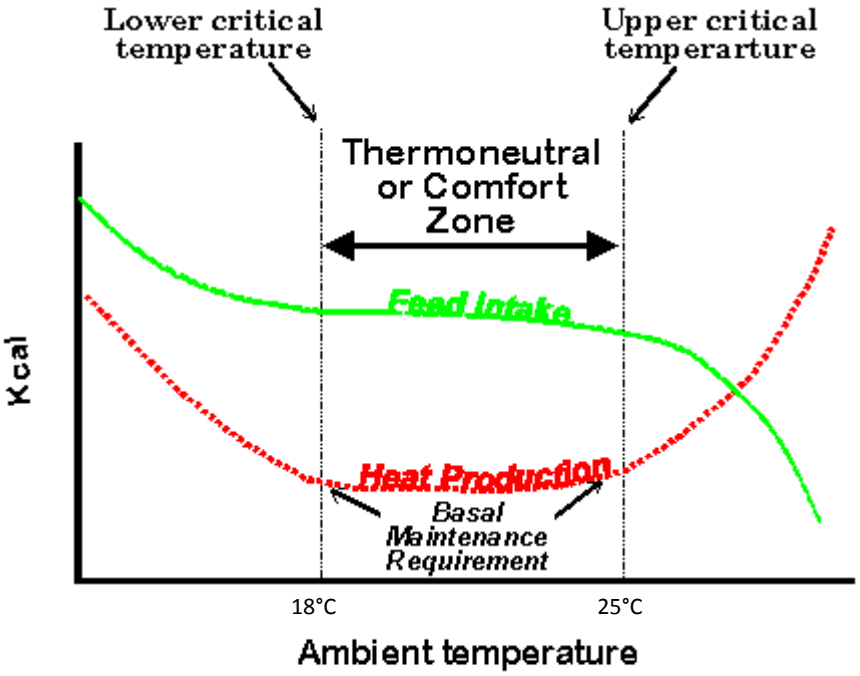


Figure 1.1. Thermo-neutral zone of pigs [adapted from (King, 2006)]

Temperature has a huge influence on growth, as does ventilation and stocking density. If the temperature is increased, growth can be reduced by as much as ~16% (Lopez, 1991). Likewise, if the stocking density is too high (too many pigs per pen), a dis-improvement in G:F is observed (Potter, 2010). These factors need to be considered when looking to improve FE, as they must be targeted in combination in order to achieve best management practices.

There are different feeder types available for pigs, and these can influence FE. There is some conflicting research, but it would appear that wet-dry feeding systems are a better option compared to dry feeding (Gonyou et al., 2000, Bergstrom et al., 2008). Feeders that are able to record weight gain, entries, intake etc. can be useful as they can reduce the time spent checking daily growths and feed intake (Young et al., 1994). However, these feeders can be expensive, labour-intensive and have a high maintenance requirement, and so may not be practical on commercial pig units.

1.3.5. Health status

While a healthy pig is able to compensate for a sub-optimal microenvironment or feeding regime, disease poses a great threat to FE. Pigs with good FE challenged with Porcine Reproductive and Respiratory Syndrome (PRRS) showed a better ability to cope with disease, compared to pigs of poorer FE (Dunkelberger et al., 2015). Furthermore, intestinal tissue from low RFI (more feed efficient) pigs challenged with lipopolysaccharide (LPS) responded better compared to high RFI pigs, indicating a better immune response, without diverting nutrients away from growth (Vigors et al., 2016a). Exposure to pathogens triggers an immune response, which will divert nutrients away from growth and maintenance in order to fuel the immune system (Patience et al., 2015), which will be discussed further in section 1.5.1.

Diseases such as PRRS, Porcine Epidemic Diarrhoea (PED), parvovirus, leptospirosis, *Ureaplasma* and *Actinobacillus* can cause severe losses to the pig industry and have a huge impact on growth performance and FE on any farm (Giles et al., 2017). The vaccination routine against these diseases is therefore an extremely necessary part of any pig herd health programme, in order to avoid performance issues and of course, mortalities (Johnson, 2008). These include stock management where ensuring adequate quarantine periods for bought-in replacement stock and treatment protocols to ensure minimal disease transfer to the established herd are followed. There are now strict biosecurity rules that farmers, producers and visitors should follow in order to keep diseases from entering the herd (Teagasc, 2014). Biosecurity measures are also important in ensuring a high health status herd so as to improve and/or maintain FE at the highest possible level.

1.4. Benefits of improving feed efficiency in pigs

Pig producers strive to improve FE in order to maximise growth, while reducing costs of production, and decreasing the emissions generated by pigs. The benefits of improving FE include those discussed below.

1.4.1. Lower production costs

The goal of every successful farming enterprise is to optimise production, whereby input costs are reduced, production is optimised and losses are at a minimum (Wilkinson, 2011). As feed currently accounts for ~70% of pig production costs (Teagasc, 2016), optimising FE should reduce this considerably, as less feed will be consumed per unit of saleable output. Similarly, low RFI animals should consume less feed to reach the same growth rates as high RFI animals (Patience et al., 2015), and this will reduce feed costs, as well as possibly shortening the production cycle, with a higher throughput of animals/fewer days to reach target slaughter weight, thereby increasing profitability.

1.4.2. Improved carcass quality

One of the benefits of improving FE is the production of higher quality pig meat. Improving FE in pigs can lead to the production of a leaner carcass, and a higher quality meat, due to better nutrient utilisation during growth (Landgraf et al., 2006). Improving meat quality is a major benefit of optimising FE, as at present, there is a high consumer demand and willingness to pay a premium for lean, tender and juicy meat.

1.4.3. Reduced environmental impact

The main emissions from pig production systems are nitrogen, phosphorus, and ammonia, which when excreted by the animal, can be harmful in the environment. It is estimated that up to 60% of nutrients are excreted undigested, and so by improving FE in pigs, more of these nutrients should be converted to (lean) meat, thereby reducing harmful emissions from pigs (Rotz, 2004).

1.5. Potential biomarkers for feed efficiency

Biomarkers for improved FE are physiological markers that are easily measured and can be used to show how an animal responds to various aspects affecting growth performance and efficiency. Quantifying these responses can be of huge benefit to the pig industry as these can be targeted to further optimise FE (Holck et al., 1998). A number of potential biomarkers for FE will now be discussed.

1.5.1. Immune markers for feed efficiency

The immune response includes two different types of immunity:

- Innate: first line of defence, defends the host against foreign microbes
- Adaptive: second line of defence, developed as the host is exposed to pathogens

Cytokines are involved in regulating both the innate and adaptive immune response of the host and production is stimulated during times of infection and disease (Murtaugh et al., 1996). The cytokines measured as part of this thesis are outlined in Table 1.3. Cytokines may be useful biomarkers for FE, as they can indicate inflammatory response and how well the host can respond to infection, and can be relatively easily measured from tissue and from blood. Previous work has found that

cytokines can be useful indicators for gut barrier function in chickens (Chen et al., 2015). Furthermore, it has been found that cytokines are linked with protein fermentation and the production of volatile fatty acids (VFAs), which indicates the need to further investigate their use as biomarkers in pigs (Pié et al., 2007).

Table 1.3. Cytokines involved in the immune response measured in this thesis [adapted from (Pond, 2001)]

Cytokine	Function	Produced by
Interleukin- 4 (IL-4)	Stimulates B-cell growth, inhibits secretion of other cytokines	Th2 lymphocytes
Interleukin- 6 (IL-6)	Promotes IL-2 production and T-cell differentiation. Stimulates acute phase response	Macrophages, T and B lymphocytes, bone marrow stromal cells, fibroblasts and keratinocytes
Interleukin- 8 (IL-8)	Activates neutrophils (chemokine), chemoattractant for neutrophils, basophils and some T-cells	Macrophages, fibroblasts, lymphocytes, granulocytes, endothelial cells, hepatocytes, keratinocytes
Tumour Necrosis factor α (TNF α)	Key mediator of inflammation, co-stimulant for lymphocyte proliferation	Macrophages

Assessing the immune system in pigs can be quite costly, and so a less expensive and easier, albeit cruder, way of investigating the immune response is to look at the haematological profile. Some of the haematological parameters studied in this thesis as potential biomarkers for FE are outlined in Table 1.4.

Table 1.4. Haemtological parameters in pigs as indicators of immune status and feed efficiency [adapted from (Mpetile, 2014)]

Parameter	Measure of/function	Indicator of
White blood cells	Number of circulating leukocytes	Response to infection
Lymphocytes	Previous pathogen invasion	Adaptive immune response
Monocytes	Response to pathogenic invasion	Innate immune response
Granulocytes	Immune response	Innate immune response
Red blood cells	Carry haemoglobin around the body	Disease/infection
Haemaglobin	Transport of oxygen	Lung and tissue function
Haemocrit	Ratio of red blood cells to total volume of blood	Health status
Platelets	Responsible for blood clotting	Low: bleeding High: blood clot in vessels

All haematological parameters should be assessed together in order to get a clear understanding of the immune response in pigs. A better understanding of the immune response in pigs may help to improve the health status of the pigs, as discussed in section 1.3.5., and therefore improve FE, as improved health will mean nutrients are utilised by the pig for growth, instead of fighting off disease. Previous work looking at the peripheral blood cell profile in healthy pigs ranked on RFI have identified potential biomarkers, including lower white blood cells, and higher red blood cells and haemoglobin in low RFI compared to high RFI pigs (Mpetile et al., 2015).

1.5.2. Serum metabolites

Metabolites present in blood can also be used to indicate health status/disease, as well as the activity of metabolic pathways in animals (Pond, 2001). Metabolites present in blood serum could also potentially be used as biomarkers for FE in pigs. Recently, serum metabolites have been measured in weaner pigs, and higher abundances of proteins suggested to have a role in nutrient utilisation and immune response, and potentially FE were observed (Grubbs et al., 2016) There are numerous tests that can be conducted on blood serum in order to measure other potential FE markers. Those which were assessed as part of this thesis are shown in Table 1.5.

Table 1.5. Blood serum metabolites in pigs (Pond, 2001, Jackson, 2007) as potential biomarkers for feed efficiency

Metabolite	Test for	Normal range[1]	Levels indicate
Total protein	Protein absorption	19-24 g/L	↓ = kidney / liver dysfunction
Blood urea nitrogen	Protein digestion and utilisation	10 – 30 mg/dL	↓ = Protein digestion reduced
Creatinine	Muscle metabolism	90-240 µmol/L	↑ = kidney disease
Creatine kinase	Indication of muscle disease (cardiac and skeletal muscle)	NR[2]	↑ = more body mass index
Triglycerides	Lipids in the blood, mobilisation of fat reserves	NR	↓ = malnutrition
Glucose	Absorption of carbohydrates from the diet, and insulin sensitivity	3.6-5.3 mmol/L	↑ = stress, hunger
Cholesterol	Synthesised in liver, adipose tissue, intestines and central nervous system	3.05-3.10 mmol/L	↑ = fat content of diet

[1] Normal range: grow-finisher pigs; [2]NR: not reported

1.5.3. Salivary cortisol

Cortisol is a corticosteroid secreted from the adrenal gland that affects metabolism of carbohydrates, lipids and protein. It follows a diurnal pattern, where concentrations are at a maximum in the morning and a minimum in the evening (Ruis et al., 1997). It has been suggested as a biomarker for FE, as animals with a higher level of serum cortisol are more likely to divert energy away from lean meat deposition and therefore have reduced FE (Richardson et al., 2004). However, in pigs, the opposite was true in one study in which pigs with low RFI (more feed efficient) had a higher level of serum cortisol (Lefaucheur et al., 2011), which may indicate that these pigs were more susceptible to stress, as suggested by the authors.

1.5.4. Gut morphology

Gut morphology in pigs can vary depending on numerous factors. Alterations are most commonly seen in weaned pigs due to the reduction in feed intake that occurs during the first few days post-weaning. Likewise, when piglets are challenged with disease, villi become shorter, as can be seen from Figure 1.2.

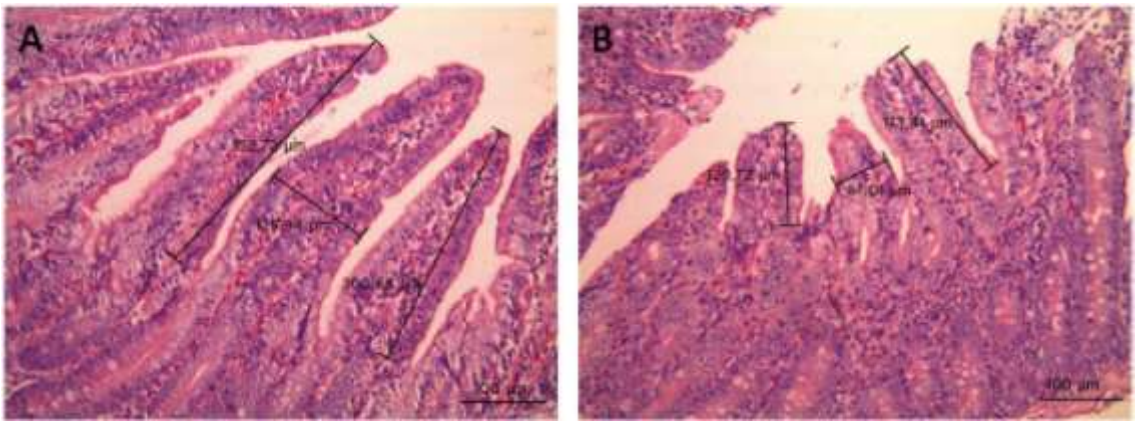


Figure 1.2. Intestinal villi of A. Healthy piglets and B. piglets challenged with lipopolysaccharide [adapted from (Parra et al., 2011)]

Histological examination usually focuses on measuring villus height and crypt depth, as it is well known that pigs with longer villi in the small intestine (mainly in the ileum) have a better nutrient absorption capacity, and therefore a better utilisation of feed (Pluske et al., 1997). Another important measure that might impact FE is the number of goblet cells. Goblet cells are found on the villi and they produce mucin, which lines the digestive tract and prevents pathogenic bacteria from adhering to the intestinal wall (Goto et al., 2012). Their presence could therefore indirectly improve FE by improving intestinal health. However, over-production of mucin can also inhibit nutrient absorption due to decreased permeability of the intestinal wall (Deplancke et al., 2001, Montagne et al., 2004). Gut morphological measures may be a useful biomarker for FE, but may be difficult to measure, as samples are usually collected after the animal has been slaughtered.

1.6. The intestinal microbiota of pigs

As in humans and other animals, the intestinal microbiota of pigs is a complex ecosystem of micro-organisms, mainly bacteria located along the gastrointestinal tract (GIT), that are in constant interaction with the host (Zhang, 2013, Pajarillo et al., 2014, Mach et al., 2015, Fouhse et al., 2016). Recently, with the advent of high-throughput sequencing techniques, numerous studies have been conducted to investigate the intestinal microbiome of pigs and, as a result, our knowledge of its composition and function is increasing (Mach et al., 2015, Zhao et al., 2015, Kim et al., 2015a). These studies will be discussed in the next sections.

1.6.1. Establishment, composition and diversity of the porcine intestinal microbiota

The GIT in pigs is essentially sterile at birth, and colonisation begins with bacteria from the birth canal, maternal colostrum and faeces, and from the surrounding environment (Mach et al., 2015, Kelly et al., 2012). The resultant intestinal microbiota comprises hundreds of different microbes that change and evolve with age, and along the length of the GIT, as shown in Figure 1.3.

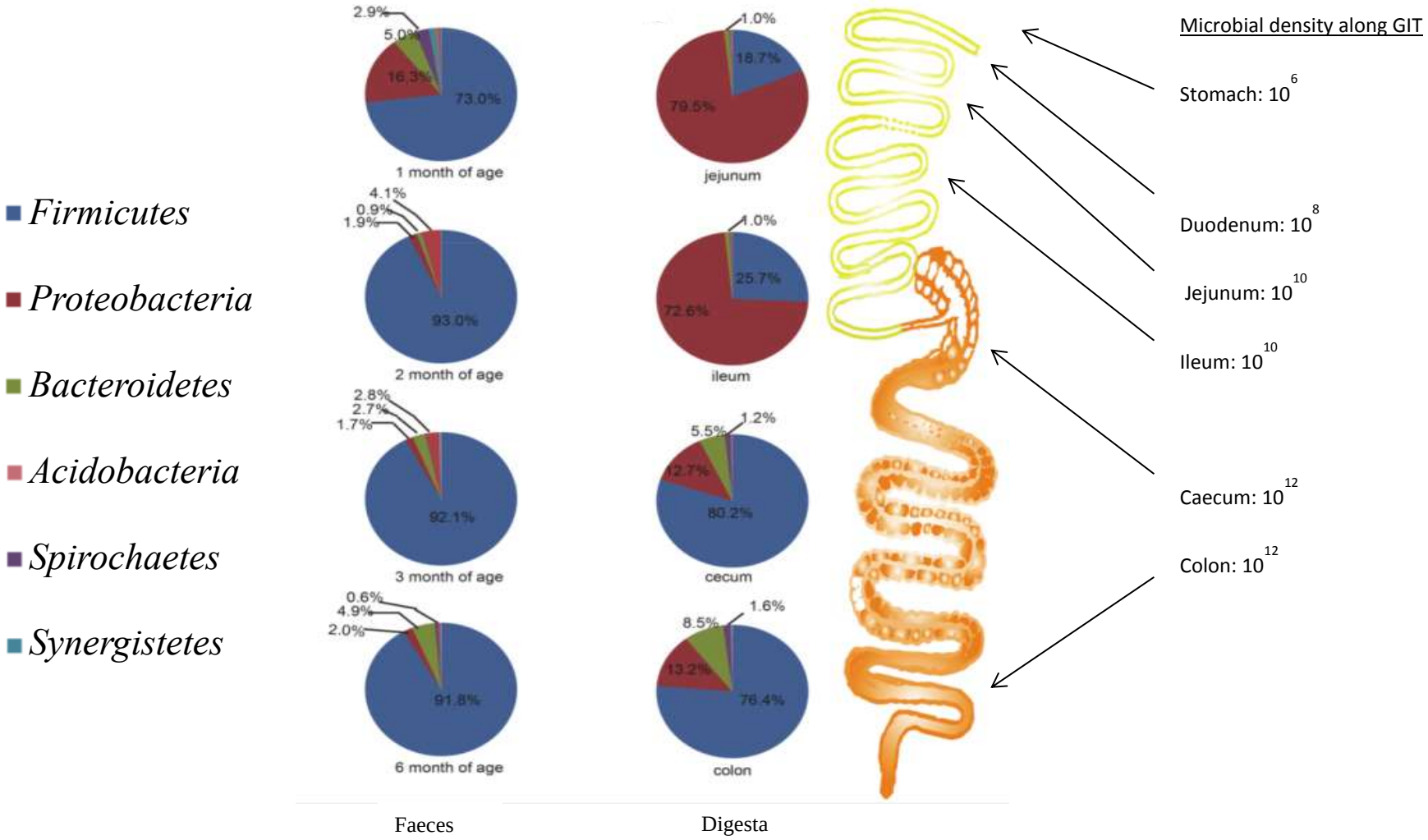


Figure 1.3. Composition of bacterial phyla throughout the pig gastrointestinal tract and changes over time as the pig ages (Zhao *et al.*, 2015, Yeoman *et al.*, 2014)

Table 1.6. Predominant bacterial taxa present in the faeces and intestinal digesta of pigs throughout the lifetime

Age[1]	Breed	Gender	Number	Sample[4]	NGS[5]	Phyla	Genera	Reference
60	Large white	Both[3]	518	Faeces	Roche 454 GS FLX Titanium	*Firmicutes* *Bacteroidetes*	*Prevotella* *Roseburia*	(Ramayo-Caldas et al., 2016)
Various	Danish French Chinese	Both	100 100 87	Faeces	Illumina HiSeq	*Firmicutes* *Bacteroidetes*	*Prevotella* *Bacteroides* *Clostridium* *Ruminococcus* *Eubacterium*	(Xiao et al., 2016)
63	Landrace x Yorkshire[2] x Duroc	Both	9 heavy 9 light	Faeces	Illumina MiSeq	*Firmicutes* *Planctomycetes* *Bacteroidetes*	*Prevotella* *Lactobacillus* *Faecalibacterium* *Prevotella* *Lactobacillus* *Bacteroides*	(Han et al., 2016)
300	Laiwu	Both	4 high back fat 4 low back fat	Jejunum Ileum Caecum	Illumina MiSeq	*Firmicutes* *Proteobacteria* *Bacteroidetes*	*Clostridium* *Escherichia* *Prevotella* *Escherichia* *Lactobacillus* *Prevotella*	(Yang et al., 2016)

Table1.6. Bacterial taxa present in the faeces and intestinal digesta of pigs throughout the lifetime (continued)

Age[1]	Breed	Gender	Number	Sample[4]	NGS[5]	Phyla	Genera	Reference
28		Male	7	Faeces		*Firmicutes*	NR[6]	(Zhao et al.,
		Female	3			*Actinobacteria*		2015)
56	Large White			Jejunum	Illumina	*Proteobacteria*		
84				Ileum	MiSeq	*Spirochaetes*		
						Proteobacteria		
168				Caecum		*Firmicutes*		
				Colon		*Firmicutes*		
						Proteobacteria		
0-21	Large White	Male	15	Faeces	Roche 454	*Firmicutes*	*Bacteroides*	(Mach et
28-70		Female	16		GS FLX	*Bacteroidetes*	*Oscillibacter*	al., 2015)
					Titanium	*Proteobacteria*	*Prevotella Oribacterium*	
						Spirochaetes	*Succinivibrio*	
						Fusobacteria		
Piglet			5	Faeces	Illumina	*Firmicutes*	*Xylanibacter*	(Kim et al.,
Grower			5		MiSeq	*Bacteroidetes*	*Lachnospiraceae* IS[7]	2015b)
Finisher			5			*Bacteroidetes*	*Prevotella*	

[1]Age is given in days; [2]yorkshire: large white; [3]Both: male and female pigs; [4]Sample type: faeces/digesta; [5]NGS: next generation sequencing platform used to identify bacterial taxa; [6]NR: not reported; [7]IS: Incertae Sedis.

As shown in Table 1.6, numerous studies have been conducted in pigs in order to determine the composition of the intestinal microbiota at different phylogenetic levels (phylum, family, and genus usually) in pigs of different ages and from different samples collected. The most abundant bacterial phyla in pigs are *Firmicutes* and *Bacteroidetes*, and as the pig ages, fluctuations in bacterial taxa occur (Figure 1.3 and Table 1.6).

Figure 1.3 and Table 1.6 also show that there are differences in the intestinal microbiota profile between the different intestinal segments. The profile of the small intestine (jejunum and ileum), for example, is very different to that of the caecum, colon and the faeces, even though data from faecal samples are commonly used as a proxy for what is present in the GIT. Differences between the faeces and the intestinal digesta are clear, with *Proteobacteria* more abundant than *Bacteroidetes*. At the genus level, there is much more variation in the composition, due to breed, age, environment etc. but many studies have reported *Prevotella*¸ *Bacteroides*, *Lactobacillus* and *Oscillibacter* as some of the most dominant genera present (Xiao et al., 2016, Kim et al., 2011, Mach et al., 2015, Yang et al., 2016).

Another important factor in the intestinal microbiome of pigs is the diversity of the bacteria present. There are different ways to measure diversity, the two most common (and the two used in the experimental chapters of this thesis) are alpha (α) and beta (ß) diversity. Alpha diversity is the measure of diversity within a sample i.e. how many different species of bacteria are present in a faecal or digesta sample (Ursell et al., 2012). It can be measured using different indices such as Shannon and Simpson, which indicate richness and abundance, and Chao1 which shows richness of bacterial species, with a focus on rare operational taxonomic units (OTUs). In humans, a higher α-diversity is linked with improved gut health (Le Chatelier et al., 2013). Although no such link has been confirmed in pigs, higher α-diversity has recently been associated

with reduced susceptibility to diarrhoea in the post-weaning period (Dou et al., 2017), and was observed in heavier body weight pigs (Han et al., 2016), which would indicate a possible link between a higher microbial diversity, and better FE. On the other hand, ß-diversity shows how similar or different samples are to each other (Ursell et al., 2012), and is a good way to see how useful faeces is as a proxy for the small and large intestine. Principal component or co-ordinate plots are commonly used to depict ß-diversity. As with α-diversity, ß-diversity of the porcine intestinal microbiota may differ due to growth, health and ultimately, FE. The microbiota of heavier pigs clusters away from that of light body weight pigs, for example (Han et al., 2016), and clustering of samples from pigs of similar fatness was found (Yang et al., 2016), which would suggest that there may be differences between the intestinal microbiota of pigs of varying FE. Studies have also found that there is distinct clustering by intestinal site; dis-similarity between the large and small intestine (Yang et al., 2016, Zhao et al., 2015), and distinct clustering by age of pig/sampling time point (Kim et al., 2011, Kim et al., 2015b), indicate the importance of collecting samples throughout the lifetime, and from the various intestinal segments in order to get a more comprehensive understanding of the porcine intestinal microbiome.

1.6.2. Role of the intestinal microbiota in pigs

The intestinal microbiota present in pigs is known to play a role in nutrient absorption and digestion as well as development and regulation of the immune system, and prevent against pathogen colonisation (Mach et al., 2015, Vigors et al., 2016a, Schmidt et al., 2011, Willing, 2010), suggesting a possible role in regulating gut structure and function. The bacteria present in the GIT work together to out-populate foreign microbes that could cause infection, by preventing their adhesion to the

epithelial lining (Fouhse et al., 2016). Bacterial members within the GIT also produce VFAs by utilising components of the diet otherwise excreted by the pig. These can be used as an extra energy source for both the colonocytes and the host (this will be discussed further in section 1.6.6.1).

1.6.3. Potential impact of the intestinal microbiota on feed efficiency in pigs

As in humans and other animals, the intestinal microbiota of pigs is known to influence a range of processes in the host, including nutrient digestion, immunity and disease resistance (Mach et al., 2015, Niu et al., 2015, Fouhse et al., 2016), all of which can influence FE, as outlined in sections 1.4 and 1.5. As a result, the potential impact of the intestinal microbiota on porcine FE cannot be ignored. Previous work has shown a strong association between bacterial genera and growth; for example, *Prevotella* was linked with higher body weights and improved ADG in growing pigs (Ramayo-Caldas et al., 2016, Mach et al., 2015). This may be attributed to the fact that *Prevotella* can produce xlyanases, mannases and ß-glucanases, enzymes necessary to break down polysaccharides present in the diet (Flint et al., 2008a). Furthermore, work conducted by Kim *et al.* showed that piglets had a higher relative abundance of *Xylanibacter* compared to older pigs, which plays a role in dietary fibre digestion (Kim et al., 2015b), and this may aid in digestion of ingredients in the post-weaning period, which may in turn improve FE if the pig can avoid the stunt in growth commonly associated with weaning.

Studies in humans have shown a clear difference in the faecal microbiota profiles between obese and lean individuals, where healthier, leaner hosts have higher bacterial diversity, indicating better gut health (Clarke et al., 2014). Obese humans have a higher relative abundance of *Firmicutes* compared to *Bacteroidetes*. Similarly,

Firmicutes was positively correlated with body weight, and negatively with *Bacteroidetes* in cloned and non-cloned pigs, used as a model for obesity (Pedersen et al., 2013).

Recently, many studies have been conducted looking at the influence of the microbiota on dietary components. For example, *Proteobacteria*, *Tenericutes*, and Candidate Division TM7, along with *Treponema*, *Methanobrevibacter* and *Campylobacter* were positively correlated with crude fibre digestibility in pigs (Niu et al., 2015). Furthermore, wheat bran fibre increased *Bifidobacterium*, and soybean fibre decreased *Lactobacillus* but increased *E. coli*, although these were measured by qPCR (Chen et al., 2014). Therefore, altering the microbiota to increase/decrease specific bacterial populations may be a useful way of improving FE, which will be further discussed in section 1.7.

A recent study conducted in pigs divergent in RFI, demonstrated that low RFI pigs (better FE) had higher numbers of *Lactobacillus* in the caecum compared to high RFI pigs, interpreted by the authors as an improvement in gut health (Vigors et al., 2016a). The pigs with better FE also tended to have increased total volatile fatty acid (VFA) concentrations in the caecum and higher proportions of butyric acid, indicating improved energy utilisation and absorption from the diet. However, as only five bacterial groups were measured by qPCR and high-throughput sequencing was not performed, the complete microbial profiles of pigs divergent in FE is, as of yet, unknown.

Further evidence that the intestinal microbiota influences obesity/leanness comes from faecal microbiota transplantation (FMT) studies (further discussed in section 1.7.2.) whereby transferring caecal microbiota from obese to germ-free mice resulted in an increase in the relative abundance of *Firmicutes* (Turnbaugh et al., 2006),

which have been shown to be higher in obese humans and pigs than in their lean counterparts. Interestingly, one group investigated the link between the microbiota and growth impairment in Malawian children, and found that when the gut of germ-free mice was colonised with faeces from growth impaired children, the mice grew significantly slower than their 'healthy' counterparts (Blanton et al., 2016). This study also found that two bacterial species *Ruminococcus gnavus* and *Clostridium symbiosum* promoted weight gain.

Further discussion of microbiota transfer between donor and recipient will be presented in section 1.7.2. Taken together, data from the studies outlined above suggests that the microbiota plays a huge role in body composition and growth, and could therefore potentially be targeted in order to improve lean meat deposition and FE in pigs.

1.6.4. Analysing the intestinal microbiota

Analysis of the microbiota in the pig GIT can be conducted in various ways. Previously, methods have been limited to culturing bacteria, but due to advances in molecular techniques over the last number of years, methods have improved drastically (Loman et al., 2012). The following sections will outline the different techniques available for examination of porcine intestinal microbiota composition and functionality.

1.6.4.1. Culture-based methods

Historically, the composition of the intestinal microbiota has been assessed using culture-based methods (Stewart, 2012), which rely on the ability of bacteria to

grow on different synthetic media under laboratory conditions. However, this is laborious and time-consuming, and the majority of bacteria present in the GIT cannot be cultured due to the inability to recreate the exact and ideal growth conditions (nutrients, temperature, pH) necessary (Stewart, 2012).

1.6.4.2. Molecular methods

Due to increased interest in the intestinal microbiota, novel, more accurate methods, which are DNA- and RNA-based, have been developed. Many of these are based on sequencing the 16S rRNA gene. Sequencing platforms began in the 1970's with Sanger (Sanger et al., 1977), followed by the first next generation sequencing platform, Roche 454, which has been superseded more recently by Illumina MiSeq and HiSeq, which are more cost-effective and higher throughput. Other sequencing platforms include Ion torrent (Life Technologies) and PacBio (Pacific Biosciences), with each platform having its own advantages and disadvantages (Loman et al., 2012). In this thesis, the V3-V4 region of the 16S rRNA gene was sequenced using the Illumina MiSeq platform.

1.6.4.3. Functional analysis of the intestinal microbiota

Assessing the functionality of the intestinal microbiota is central to understanding the potential effects that the microbiota can have on the host. There are a number of different approaches used in order to achieve this, some of which will be outlined here. Compounds can be measured that are produced by the bacteria present in the GIT, and used as an indicator of functionality.

- **Beneficial:** VFAs are produced along the porcine GIT (mainly in the caecum) by microbial fermentation of undigested carbohydrates and fibre. The main VFAs produced are acetate, propionate and butyrate. These can supply the pig with 11-25% of the total energy requirements for maintenance and production, and are a source of energy for epithelial cells. Their production also results in reduced pH and therefore a potential reduction in colonisation of potential pathogens (Bergman, 1990, Williams, 2001). Specific bacterial members of the intestinal microbiota have been implicated in the production of certain VFAs; for example, species of *Ruminococcus* and *Lachnospiraceae* from *Firmicutes* produce butyric acid and members from *Bacteroidetes* such as *Prevotella* and *Bacteroides* produce propionic acid (Louis et al., 2017)
- **Harmful:** toxic compounds, such as biogenic amines, NH_3 (ammonia), volatile indoles and phenols, are produced by bacteria during protein fermentation. These are a source of nitrogen and can therefore affect FE of the animal, adding an energy cost to the host through the need to excrete the excess substances produced, such as urea (Karovicova et al., 2003).

While determination of bacterial metabolites in the intestine provides valuable data, information can be limited due to the accuracy of the analyser and laborious detection methods. Other methods (e.g. metabolomics) can overcome this, and could therefore improve our knowledge of the intestinal microbiota and its potential link with FE.

Other methods of assessing microbial functionality include:

- **Whole-genome analysis:** this involves metagenomic analysis (down to species level) of the potential function/functional capacity of the microbiota. Large amounts of DNA can be sequenced using Shotgun sequencing (using e.g. the

Illumina HiSeq platform), but it is quite expensive. This approach involves cleaving the DNA into smaller fragments to allow for successful sequencing of the genomes, and uses computer programming to overlap the reads in order to assemble them into a continuous sequence to use for analysis (Venter et al., 2001)

- **Transcriptomics:** measures RNA transcripts that are present in the bacterial cell at a certain time point. It involves the isolation of RNA from the microbiota followed by complementary DNA synthesis and sequencing. However, due to the unstable nature of RNA, this method can be complicated (Poroyko, 2012)
- **Proteomics:** not sequence-based as with other methods above, but involves the study of proteins present in bacteria at a certain time point (Cox, 2007) and has been used in pigs to investigate gut health in pigs (Bendixen et al., 2011)

Given that functional analysis can be very expensive, there are now a number of ways to predict the metabolic pathways that the intestinal microbiota are involved in using software tools such as PICRUSt, FragGeneScan, MetaGeneMark and Glimmer-MG. These databases are based on the data generated from sequencing the human metagenome, where the 16S rRNA gene sequencing data is used to give an indication of potential function. The KEGG database, among others, contains a list of biological functions which can be used to help identify possible functions of the bacteria present, where reference genomes are used to assign relative abundance. This database also applies to high-throughput sequencing platforms (Kim et al., 2015c).

1.7. Manipulating the intestinal microbiota, with a view to optimising feed efficiency in pigs

Due to the important role that the intestinal microbiota plays in nutrient digestion and host health, as discussed in this review, optimisation of the intestinal microbiota is a very topical area. With this in mind, differences in the bacterial profile in pigs of good versus poor FE, mainly at genus or indeed species level, could be targeted to optimise FE e.g. the abundance of *Bacteroides*, which is known to aid carbohydrate digestion, and *Oscillibacter*¸ which provides health benefits to the host could be increased (Mach et al., 2015), among others. However, targeted manipulation of the intestinal microbiota may prove challenging due to the limited knowledge of the "optimum" bacterial profile for FE.

There are three main ways to alter the intestinal microbiota of pigs:

- Antibiotic treatment i.e. in-feed medication used as growth promotor
- Dietary intervention strategies i.e. administration of feed additives
- Inoculation with live bacteria

In the following section, prebiotics are the focus of the discussion on feed additives, as this was the type of additive used in one of the experimental chapters of this thesis.

1.7.1. Feed additives, with a focus on prebiotics

The use of feed additives can be an effective way of improving animal production and health status. As the main objective of this thesis is to improve FE, feed additives can be a useful way of achieving this. They have become increasingly popular, especially since the 2006 ban on the routine use of in-feed antibiotics in the European Union. Pigs fed diets containing antibiotics grew faster than animals without, and required around 10% less feed to achieve this (Chattopadhyay, 2014).

Enzymes, such as xylanase and ß-glucanase, are added to improve nutrient availability from the diet (de Lange et al., 2010, Garry et al., 2007, Torres-Pitarch et al., 2017). Organic acids, mainly added to weaner diets, work by reducing the pH of the stomach, thereby reducing harmful bacteria present, and improving digestion (Partanen, 1999, Mani-López et al., 2012). Essential oils such as thymol and cinnamaldehyde are added to the diet to improve gut health, as they enhance the immune system due to their antimicrobial capabilities (Li et al., 2012). Flavours (e.g. vanilla) and sweeteners (e.g. saccharin, talin) can be added to weaner diets also and by ingesting solid feed, the digestive system matures faster in terms of gut structure, digestive enzyme levels etc (Sterk, 2008, Sulabo, 2010). However, there have been inconsistent findings with regard to the efficacy of feed additives.

Probiotics (live bacteria fed to the host), are another group of commonly used feed additives; however, these will be discussed in Section 1.7.2.1.

A prebiotic was first defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson et al., 1995), but has been updated to "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (Roberfroid, 2007).

For an ingredient to be considered a prebiotic, it must be resistant to digestion in the upper GIT, a selective substrate for good bacteria, and induce health benefits to the host. All carbohydrates can be prebiotics, but there are only three "true" prebiotics; inulin and oligofructose, galactooligosaccharides, and lactulose (O'Sullivan et al., 2010). As inulin was the prebiotic used in this thesis, it will be the only prebiotic discussed here. Inulin is a non-digestible dietary fibre derived mostly from chicory,

which belongs to a carbohydrate group called fructans. It is made up of several sugars linked together, i.e. it is an oligosaccharide (chemical structure shown in Figure 1.5). The structure of inulin can be either linear or branched, and chain length or degree of polymerisation (DP) can vary between 2 to 65 units (Yasuda, 2007).

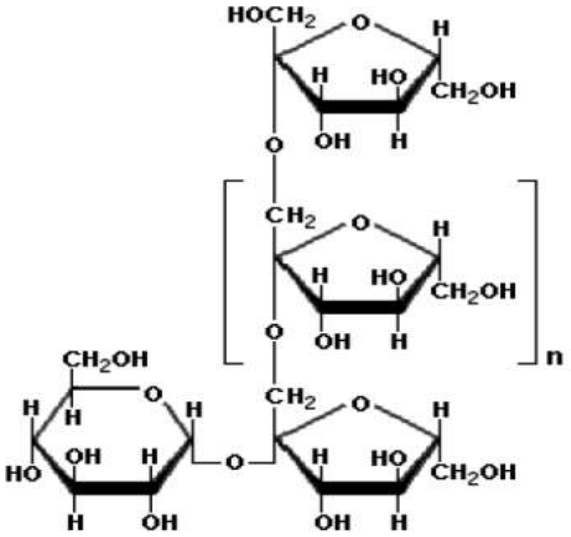


Figure 1.4. The chemical structure of inulin [adapted from (Yasuda, 2007)]

Short-chain inulin (low molecular weight) is broken down more quickly compared to long chain inulin (high molecular weight), and both chain lengths should bypass the upper GIT undigested and be fermented in the large intestine by the microbiota present (Kelly, 2008). However, some previous findings suggest that fermentation of short chain inulin can occur in the small intestine also (Yasuda et al., 2007). Table 1.7 outlines findings from studies that have included inulin in the diet of pigs. Results vary regarding improvements in intestinal microbiota profile, and FE.

Table 1.7. Studies conducted in pigs where inulin was included in the diet

Growth phase	Inclusion rate & duration	Effect on growth	Other effects	Effect on intestinal microbiota	Reference
Suckling pigs	20 g/Kg aqueous inulin 20 g/Kg aqueous-alcoholic inulin 40 g/Kg dried artichoke 40 g/Kg dried chicory 10-84 days of age	Artichoke or chicory root ↑ weight gain	Plasma cholesterol ↓ in all groups vs. control Immunoglobulin A & G ↑ in aqueous inulin fed groups	Not determined	(Grela et al., 2014)
Weaners	Iron-deficient diet + 2% vs. 4% Synergy 1[1] 5 wks	No difference	↑ Hb[5] in pigs fed 4% vs. 2% ↓ sulfide concentration in colon of pigs fed 4% (sulfide is a putrefactive agent)	Not determined ↓ sulfide in colon digesta suggests modified microbial population	(Yasuda et al., 2006
Weaners	Iron-deficient diet + 4% Synergy 1 6 wks. (Exp. 1) 8 wks. (Exp. 2)	Not reported	Inulin detected in stomach, jejunum & ileum, not in caecum or colon ↑ fructose concentrations in stomach & upper jejunum – better iron absorption	Not determined but higher enzyme activity in the caecum	Yasuda et al., 2007)
Weaners	Iron-deficient diet + 4% Synergy 1 6 weeks	No difference	↑ Hb concentrations ↑ mucin gene expression in duodenum but not colon	↑ *Bifidobacterium* and *Lactobacillus* in caecum No effect on *E. coli* or *Clostridium*	(Tako et al., 2008)

Table 1.7. Studies conducted in pigs where inulin was included in the diet (continued)

Growth phase	Inclusion rate & duration	Effect on growth	Other effects	Effect on intestinal microbiota	Reference
Weaners	1.5 % inulin (no additional zinc) Long chain (HP[4]) 2 weeks	Not reported	No effect on manganese & zinc absorption Liver copper ↑ in pigs fed inulin- higher absorption in inulin	Not determined	(Untea et al., 2013)
Finishers	Low P[2] diet High P diet Low P diet + 2% inulin High P diet +2% inulin 51-95 kg	Not reported	Inulin: ↑ total VFAs[6] & ↑ butyric acid in caecum & proximal colon & no effect on P or Ca[7] metabolism	Inulin: ↓ *Enterobacteriaceae* in colon No effect on digesta pH or ileal bacteria No difference in *Lactobacillus* or *Bifidobacterium* in LI[8]	(Varley et al., 2010)
Finishers	High CP[3] +1.25% inulin Low CP + 1.25% short chain 74-105 kg	Not reported	Inulin: ↑ faecal Nitrogen(N) (better N utilisation)	↑ *Bifidobacterium* in caecum with inulin ↓ populations of *E. coli* in pigs fed high CP + inulin	(Lynch et al., 2007)

[1]Synergy1: 50:50 mixture of long and short chain inulin. [2]P: Phosphorous. [3]CP: Crude protein. [4]HP: long chain inulin. [5]Hb: Blood haemoglobin. [6]VFAs: Volatile fatty acids. [7]Ca: Calcium. [8]LI: Large intestine.

With regard to the prebiotic inulin, other studies have been conducted apart from those detailed in Table 1.7, with most generating contradictory results (Awad et al., 2013, Frantz et al., 2003, Grela et al., 2014, Huang et al., 2015, Loh et al., 2006, Pierce et al., 2005). Interestingly, when inulin was fed to pregnant and lactating sows, bacterial populations of *Enterobacteria* were lower in the stomach and *Clostridium leptum* were higher in the caecum of subsequent offspring (Paßlack et al., 2015) highlighting the maternal influence on pig growth and the intestinal microbiota, which may be a useful way of manipulating subsequent microbiota in order to improve FE. As inulin is not entirely degradable by the host, it has been suggested to increase the abundance of bacterial populations that play a central role in the fermentation of dietary polysaccharides in the hindgut (O'Sullivan et al., 2010). This often results in increased production of VFAs, particularly butyric acid, which can be used by the host, as well as aiding the immune system by increasing beneficial bacteria and their immune stimulating products, which should lead to an improvement in intestinal health and growth in pigs (Kozłowska et al., 2016).

1.7.2 Inoculation of live bacteria

1.7.2.1. Probiotics

Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). There are a number of potential probiotics available for pigs e.g. strains of *Bacillus*, *Lactobacillus*, *Enterococcus* (Casey et al., 2007, Prieto et al., 2014, Chaucheyras-Durand et al., 2010). Improvements in FE, health status and VFA production have been observed, but are very much strain-dependent (Prieto et al., 2014, Veizaj-Delia, 2010). Probiotics can impact the porcine intestinal microbiota also, with reductions in potentially pathogenic bacteria, such as members of *Proteobacteria*, as well as enhancement of beneficial genera such as

Oscillibacter and *Lactobacillus* (Pajarillo et al., 2015, Riboulet-Bisson et al., 2012). As with the other dietary additives discussed in section 1.7.1, there are some conflicting results with regard to improving FE and increasing the numbers of beneficial bacteria. Probiotics will not be discussed in depth in this review as they were not used in any of the experimental chapters.

1.7.2.2 Faecal microbiota transplantation (FMT)

Faecal microbiota transplantation (FMT) or faecal bacteriotherapy involves altering the microbiota present in the GIT to a more beneficial profile by transferring desirable microbiota from a donor(s) to a recipient. It aims to add/supply microbes to the GIT that will improve gut health and function (Borody et al., 2012) or restore intestinal homeostasis. Intestinal disorders in humans, most notably recurrent *Clostridium difficile* infection, have been effectively treated using FMT. The first known faecal transplant was performed in humans in the 4th century in China and was used for a variety of reasons to benefit the recipient (Nieuwdorp, 2014). Successful establishment of the new gut microbiome in the recipient following FMT may be of concern, but FMT could be a useful tool in pig production as it could provide the complete spectrum of "optimum" microbes needed for improved FE, especially when compared to additives such as pre/probiotics and antibiotics (Borody et al., 2014).

There are two main ways to deliver faecal microbiota from donor to recipient: oral consumption and rectal administration, with the latter more commonly used in humans. Early intervention/transplantation of microbiota is necessary (due to the pig gut being almost completely sterile at birth) and multiple inoculations may be required to establish and maintain the microbiota in the GIT (Schmidt et al., 2011). While it may appear to be a promising technique, there are some limitations, such as lack of

established protocols, as well as issues with survivability of bacteria present in the inoculum and the transfer of pathogens or other undesirable microorganisms. Preparation and storage of the faecal inoculum is critical, as microbes present in the faecal material can be greatly affected when frozen and thawed, with potential for cell damage and death. The latter can be prevented with the use of cryoproctectants (glycerol, saline) which protect bacteria during cell freezing, and improve viability at thawing (Guerin-Danan, 1999, Waite et al., 2013, Borody et al., 2014). Recently, it was found that that there were no differences between storage conditions in terms of the bacterial populations from human faecal samples (fresh, frozen at -80°C, snap frozen and then stored at -80°C), with the individual sample having more of an impact on the bacterial populations present than the storage method (Fouhy et al., 2015).

Table 1.8. Summary of faecal microbiota transplantation (FMT) studies performed to date where pigs were used either as the donor or the recipient

Donor/recipient	Numbers	Treatments	Results	Reference
Pig to pig	5 donor pigs per breed to 6 pigs per group	Group 1 & 2: pigs receiving Yorkshire microbiota Group 3 & 4: pigs receiving Tibetan microbiota Group 5 & 6: pigs receiving sterile PBS[3] 10 mL given every day for 3 days, then every second day (days 4-15) and 20 mL every 5 days (days 16-48) Acute colitis induced via destran sulphate sodium	Successful implantation of donor microbiome in recipients. Yorkshire-associated recipients had a higher gene expression of colonic TLR4 and 8 when acute colitis was induced. Tibetan breed may therefore have better disease resistant capabilities	(Xiao et al., 2017)
Pig to pig	5 donors 58 preterm pigs	Homogenised colon content from 14 day old piglets (10^9 CFU twice daily) on day 1 and 2 Pigs euthanized on day 5	Diarrhoea score tended ↓ in FMT piglets FMT ↓ necrotising enterocolitis lesions Lactase, maltase ↑ Higher mortality rate in FMT piglets	(Martin et al., 2015)[4]
Humans (10 yr old boy) to piglets[1]	1 boy 28 piglets	Faeces from boy given orally to piglets from 12 h old, 1 mL/day for 3 days & every other day until 10 days old	By 5 days old, human microbiota were established in the pig gut and became stable at 12 days old	(Pang, 2007)

Table 1.8. Summary of faecal microbiota transplantation (FMT) studies performed to date where pigs were used either as the donor or the recipient (continued)

Donor/recipient	Numbers	Treatments	Results	Reference
Humans to piglets[2]	10 humans 1 infant 36 pigs	Faecal inoculum administered orally Microbial population monitored for 35 days	Human microbiota easily colonises the pig GIT, indicating that pigs can be used as a model for the human GIT	(Zhang, 2013)
Pigs to germ free mice	5 pigs/breed 8 mice/treatment	Faeces from 5 pigs/breed and transplanted into mice (intra-gastric and on fur) for 5 weeks	Bacterial profile and intestinal structure detected in pigs are their mice recipients	(Diao et al., 2016)
Pigs to germ/pathogen-free mice	-	Faecal samples taken from either 20 or 60 day old pigs given orally to germ-free mice	Microbiota of pig successfully grafted into mice GIT	(Hirayama, 1999)

[1]germ-free: born by caesarean; [2]born via caesarean into sterile environment; [3]Sterile phosphate buffered saline (PBS) given as a control treatment; [4]Conference abstract.

Studies where pigs were used either as the donor or recipient of FMT are outlined in Table 1.8, with the latter being more commonly evaluated to date. All of the studies agree that the faecal inoculum given was easily established in the recipient, and that the bacterial profile of the recipient matched that of the donor. There have been many studies conducted in humans and rodents that have proven that FMT will transfer traits from donor to recipient, including obesity (Alang et al., 2015, Ridaura et al., 2013) and behaviour (Collins et al., 2013) This indicates the potential to deliver a faecal inoculum containing bacteria deemed optimal for improved FE, as a novel means of improving FE in pigs.

To date, FMT has not been conducted in pigs with the aim of improving FE, and in fact, only one conference abstract has reported pig-to-pig FMT; it successfully treated necrotising entero-colitis, but resulted in increased mortality in piglets (Martin et al., 2015).

1.8. Conclusions

As feed accounts for >70% of the costs of pig production, ways are continuously being sought by which to improve FE in pigs. Genetics, diet, health and management factors, as well as rearing environmental all have an influence on FE. While all of these are contributing factors, we cannot forget the impact that the gut microbiota has on the pig. The intestinal microbiota is considered an organ in itself, and makes a huge contribution to growth and performance, as well as regulating nutrient utilisation, and the immune response, and potentially FE. Ways of manipulating the microbiota in order to establish a more optimal microbial profile (e.g. feed additives, FMT) may be a useful means of improving FE.

The literature reviewed here, indicated a gap in the knowledge in certain areas relating to improving FE in pigs through intestinal microbiota-based approaches. With this in mind, the aims of this PhD thesis are to further the knowledge of the intestinal microbiota present in pigs of varying FE, and to investigate strategies to manipulate the microbiota present in order to improve FE.

1.9. Overall objectives of the research

- To characterise the intestinal microbiota, intestinal structure and function, immunology and blood parameters in pigs divergent in FE, across different rearing environments

- To evaluate the use of FMT as a strategy to alter the intestinal microbiota in pigs, in order to improve FE

- To investigate dietary strategies, i.e. feeding a prebiotic, inulin, to promote the proliferation and persistence of the optimum microbial profile in pigs, in relation to improved FE

2. Exploring a possible link between the intestinal microbiota and feed efficiency in pigs

UM. McCormack, T. Curião, SG. Buzoianu, ML. Prieto, T. Ryan, P. Varley, F. Crispie, E. Magowan, BU. Metzler-Zebeli, D. Berry, O. O'Sullivan, PD. Cotter, GE. Gardiner, PG. Lawlor. 2017. Exploring a possible link between the intestinal microbiota and feed efficiency in pigs.

Appl Environ Microbiol doi:10/1128/AEM.00380-17.

2.1. Abstract

Feed efficiency (FE) is critical in pig production for both economic and environmental reasons. As the intestinal microbiota plays an important role in energy harvest, it is likely to influence FE. Therefore, our aim was to characterize the intestinal microbiota of pigs ranked as low, medium and high residual feed intake (RFI; a metric for FE), where genetic, nutritional and management effects were minimized, in order to explore a possible link between the intestinal microbiota and FE. Eighty one pigs were ranked on RFI between weaning and day 126 post-weaning, and 32 were selected as the extremes in RFI (12 low, 10 medium, 10 high). Intestinal microbiota diversity, composition and predicted functionality were assessed by 16S rRNA gene sequencing. Although no differences in microbial diversity were found, some RFI-associated compositional differences were revealed, principally among members of *Firmicutes*, and predominantly in faeces at slaughter (albeit mainly for low abundance taxa). In particular, microbes associated with a leaner and healthier host (e.g. *Christensenellaceae, Oscillibacter, Cellulosilyticum*) were enriched in low RFI (more feed efficient) pigs. Differences were also observed in the ileum of low RFI pigs; most notably *Nocardiaceae* (*Rhodococcus*) were less abundant. Predictive functional analysis suggested improved metabolic capabilities in these animals, especially within the ileal microbiota. Higher ileal isobutyric acid concentrations were also found in more efficient pigs. Overall, differences observed within the intestinal microbiota of low RFI pigs compared to their high RFI counterparts, albeit relatively subtle, suggest a possible link between the intestinal microbiota and FE in pigs.

2.2. Importance

This study is one of the first to show that differences in intestinal microbiota composition, albeit subtle, may, at least in part, explain improved FE in low RFI pigs. One of the main findings is that, although microbial diversity did not differ among animals of varying FE, specific intestinal microbes could potentially be linked with porcine FE. However, as the factors impacting FE are still not fully understood, intestinal microbiota composition may not be a major factor determining differences in FE. Nonetheless, this work has provided a potential set of microbial biomarkers for FE in pigs. Although culturability could be a limiting factor, and intervention studies are required, these taxa could potentially be targeted in the future, in order to manipulate the intestinal microbiome so as to improve FE in pigs. If successful, this has the potential to reduce both production costs and the environmental impact of pig production.

2.3. Introduction

Feed accounts for ~70% of the total cost of producing a pig (Teagasc, 2016). Therefore, improving feed efficiency (FE) will increase profitability, while also reducing the environmental impact of pig production (Rotz, 2004). The porcine intestinal microbiota is considered an important "organ" with a crucial role to play in nutrient processing and harvesting of ingested energy (Ramayo-Caldas et al., 2016, Xiao et al., 2016, Fouhse et al., 2016). It is therefore plausible to suggest that the porcine intestinal microbiota could potentially be targeted to improve FE. Indeed, porcine metabolism is impacted by the complex interplay between the resident intestinal microbes, their metabolites e.g. volatile fatty acids (VFAs), and enterocyte function (Willing, 2010, Kennelly et al., 1981, Liu, 2015, Shirkey et al., 2006). Microbial mechanisms of potential relevance to FE include positive feedback between certain microbes and mucin production, by goblet cells along the villi, and through up-regulation of butyric acid production (Wlodarska et al., 2015, Burger-van Paassen et al., 2009). Interestingly, studies in cattle have shown differences in the intestinal microbiota in animals differing in FE (Myer et al., 2015, Carberry et al., 2014). However, very few studies to date have explored the possible link between the intestinal microbiota and FE in pigs.

In recent years, the pig microbiome has become the focus of much attention (Vigors et al., 2016a, Pedersen et al., 2013, Frese et al., 2015, Buzoianu et al., 2012b, Metzler-Zebeli et al., 2015b). The porcine intestinal microbiota is dominated at the phylum level by *Firmicutes*, *Bacteroidetes* and *Proteobacteria* (Ramayo-Caldas et al., 2016, Xiao et al., 2016, Kim and Isaacson, 2015a, Zhao et al., 2015). Differences within the intestinal microbiota have explained variability in body weight in pigs; for example, at the phylum level, *Firmicutes* and *Planctomycetes* have been found at higher relative abundance in heavier pigs, while *Bacteroidetes* were more abundant in lighter pigs (Han

et al., 2016). In the same study, body weight-associated differences were found at the genus level (Han et al., 2016) and a study by Mach *et al.* (2015) showed that *Prevotella* was positively correlated with body weight (Mach et al., 2015). Intestinal microbiota composition has also been shown to vary between lean and obese pigs, with an increased abundance of *Firmicutes* found in obese pigs (Pedersen et al., 2013). In addition, bacterial diversity within the intestinal tract has been found to be higher in pigs with heavier body weights and improved growth rates (Ramayo-Caldas et al., 2016, Han et al., 2016). However, to our knowledge, only one study to date has investigated the association between FE in pigs and the intestinal microbiota (Vigors et al., 2016a). It demonstrated an increased abundance of *Lactobacillus* in the caecum of more feed efficient pigs; however, only *Firmicutes, Bacteroidetes, Bacteroides, Lactobacillus* and *Enterobacteriaceae* were measured (by quantitative PCR). Pigs with better FE also tended to have higher concentrations of total VFAs in the caecum and butyric acid in the colon, which may be explained by differences in microbial composition and function (Vigors et al., 2016a, Vigors et al., 2016b).

Characterizing the intestinal microbiota of highly feed efficient pigs could help to define an "optimal" microbial profile for improved FE. Shifts in microbial community structure associated with FE might suggest opportunities to modulate the intestinal microbiota composition in order to improve FE. In particular, the enrichment of specific microbes, supported by beneficial functionality could pinpoint prospective microbial biomarkers for FE within the porcine intestinal microbiota. "Optimization" of the microbiota could then potentially be achieved through the use of these specific bacterial taxa as probiotics, or alternatively by increasing their abundance via the use of prebiotics or other dietary supplements (Wlodarska et al., 2015, Hou et al., 2015, Prieto et al., 2014) or by faecal microbiota transplantation (de Vos, 2013).

Therefore, in the present study, we investigated the hypothesis that the composition and potential functionality of the intestinal microbiota is linked with FE in pigs. The objective was to determine if there were any differences in microbial diversity and/or relative abundance of bacterial taxa, at the phylum, family and genus level, within the faecal microbiota throughout the life of the pig, and in the ileal and caecal microbiota at slaughter (at ~166 days of age), in pigs ranked based on residual feed intake (RFI; a metric for FE).

2.4. Materials and Methods

2.4.1. Ethical approval

The pig study was approved by the animal ethics committees of Teagasc (TAEC9/2013) and Waterford Institute of Technology (13/CLS/02) and performed according to European Union regulations outlining minimum standards for the protection of pigs (91/630/EEC) and concerning the protection of animals kept for farming purposes (98/58/EC). An experimental license (number AE1932/P004) was obtained from the Irish Health Products Regulatory Authority (HPRA).

2.4.2. Animal management and sample collection

A schematic illustration depicting animal management, selection and sample collection is shown in Figure 2.1. Multiparous F1 sows (Large White x Landrace; Hermitage Genetics, Kilkenny, Ireland) were selected at weaning and randomly inseminated using semen from one of five Hylean Maxgro boars (Hermitage Genetics). At farrowing, piglets were tagged for identification purposes and weighed. Litters were kept intact between farrowing and weaning but, when necessary for welfare reasons, surplus/non-thriving pigs were fostered to non-trial sows. At weaning, 7 litters comprising male and female pigs, each with 11 to 12 pigs were selected, so that a total of 81 pigs (44 males and 37 females) were blocked by litter ancestry and randomly assigned to individual weaner pens (1.2 m × 0.9 m) with plastic slats (Faroex, Manitoba, Canada) and solid plastic dividers between pens. On day 42 post-weaning (pw), pigs were transferred to individual finisher pens (1.81 m × 1.18 m; fully slatted with solid plastic panel partitions) and remained there until the end of the study. Feed was available *ad-libitum* as dry pellets *via* stainless steel dry feed hoppers, 30 cm in length (O'Donovan Engineering, Co. Cork, Ireland). The ingredient and chemical composition

of the diets fed are shown in Table S2.1. Any pigs treated with antibiotics were removed from the study.

Individual faecal samples were collected following rectal stimulation at weaning and at days 42 pw and 138 pw (day prior to slaughter at the end of the finishing period), immediately snap- frozen in liquid nitrogen and stored at -80 °C for microbiota and VFA analysis.Individual body weight and feed disappearance were manually recorded every 2 weeks from weaning up to day 126 pw and used to calculate performance indicators [average daily feed intake (ADFI), average daily gain (ADG) and feed conversion efficiency (FCE)]. Ultrasonic back fat and muscle depth measurements were recorded using a Piglog 105 ultrasound scanner (Carometec, Herley, Denmark) on the same day as weighing, between days 42 and 126 pw. Back fat and muscle depth were measured between the 3rd and 4th last lumbar vertebrae, 7 cm from the midline for calculation of lean meat content.

On day 126 pw, extremes for RFI (the metric used for FE in the present study) were selected on the basis of measurements calculated from weaning for each pig. Residual feed intake measures the difference between actual and expected feed intake, where the expected feed intake is based on live-weight, rate of gain, body fat and muscle content of the individual pig (Patience et al., 2015). It was calculated as the residuals from a least squares multiple-regression model of ADFI on ADG, metabolic live weight, sex and all relevant two-way interactions, as well as the effects of back-fat and muscle depth. Pigs were ranked, within litter, on RFI (low, medium and high, where low RFI pigs are the most feed efficient), so that a minimum of two standard deviations in RFI existed between the mean of the low and high RFI pigs within litter. Thirty-two pigs were selected [low RFI (n= 12), medium RFI (n= 10), and high RFI (n=10)], and samples from these ranked pigs were used in all subsequent analyses.

As outlined above, pigs were on test between weaning and day 126 pw (to represent the normal productive life of the pig in Ireland). However, mean live weight at day 126 pw was ~129 Kg which was higher than originally predicted for this age. Following the test period, selection of extremes in RFI was undertaken, as outlined above, and, as this took time, all pigs were slaughtered two weeks later, on day 139 pw (corresponding to ~166 days of age) by CO_2 stunning followed by exsanguination. Hot carcass weight was recorded immediately following slaughter and was multiplied by 0.98 to obtain cold carcass weight. Kill-out percentage was calculated as [(carcass weight/body weight at slaughter) × 100]. Back-fat and muscle depth were measured at 6 cm from the edge of the split back at the third and fourth last ribs using a Hennessy Grading probe (Hennessy and Chong, Auckland, New Zealand). Lean meat yield was estimated according to the following formula: Lean meat yield = $60.30 - 0.847\ X_1 + 0.147\ X_2$ [where X_1= back-fat depth (mm) and X_2= muscle depth (mm)]. Immediately after slaughter, the heart, kidneys, liver, and lungs were collected, trimmed of fat, blood clots removed, blotted dry and weighed. The stomach was emptied of contents, flushed with water and blotted dry before being weighed. Digesta samples were collected from the 32 selected pigs from the terminal ileum (15 cm proximal to the ileo-caecal junction) and from the terminal tip of the caecum. Samples were immediately snap-frozen in liquid nitrogen and stored at -80 °C for subsequent microbiota and VFA analysis. Ileal tissue samples (~3 cm sections) were collected, rinsed in phosphate buffered saline (PBS) and placed in No-Tox fixative (Scientific Device Lab, Des Plaines, IL, USA) on a shaker for 48 h. The samples were then stored at room temperature until histological analysis. After sampling, the small intestine was emptied of contents, flushed with water, trimmed of connective tissue, blotted dry and weighed.

2.4.3. Salivary cortisol analysis

Cortisol concentrations were determined in saliva samples collected in the days prior to slaughter (days 135 and 138 pw) in duplicate using a high sensitivity enzyme linked immunosorbent assay (ELISA) kit (Salimetrics, Europe Ltd, Suffolk, UK) according to the manufacturer's instructions.

2.4.4. Histological analysis of ileal tissue

Fixed ileal tissue samples were dehydrated through a graded alcohol series, cleared with a Sub-X clearing agent (Surgipath, Richmond, IL, USA) and embedded in paraffin wax. Tissue samples were sliced using a microtome (Leica RM2235, Wetzlar, Germany), mounted on a microscope slide and stained with haematoxylin and eosin (Sigma-Aldrich, St. Louis, MO, USA) for histological analysis. Ten villi per slide were examined for villus height, villus width, crypt depth and number of goblet cells under a light microscope at ×400 magnification.

2.4.5. 16S rRNA gene amplicon sequencing of faecal and intestinal microbiota

Total DNA was extracted from faecal, ileal, and caecal samples using the QIAamp DNA stool minikit (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions, apart from adding a bead beating step after sample addition to the InhibitEX buffer, and increasing the lysis temperature to 95 °C to increase DNA yield (Buzoianu et al., 2012a).

Microbial profiling was performed using high-throughput sequencing of the V3-V4 region of the 16S rRNA gene (paired-end reads of 300 bp or 250 bp) on an Illumina MiSeq platform following the standard Illumina protocol, except that the PCR mix volume was doubled in the first PCR step, and 30 cycles were used instead of 25 (Fouhy et al., 2015). Any samples with less than 40,000 post-quality reads were removed from the analysis. Raw sequences were merged using Flash (with a minimum overlap of 30

bp and minimum read length of 460 bp) and quality checked using the split libraries script (with default parameters) from the QIIME package version 1.9.1. Reads were clustered into operational taxonomic units (OTUs) using *de novo* picking, with a 97% sequence identity threshold and chimeras and singletons removed with the 64-bit version of USEARCH (version 7) (Edgar, 2010). Subsequently, OTUs were aligned to the SILVA rRNA specific database (version 111) to assign taxonomy and a phylogenetic tree was generated within QIIME. Alpha-diversity indices i.e. Chao1 (which measures richness based on rare OTUs) and Shannon and Simpson (which measure richness and evenness) and ß-diversity analyses were also calculated within QIIME, again using a rarefaction level of 97% identity. Principal coordinate analysis (PCoA) plots, based on unweighted Unifrac distances, were visualized using EMPeror v0.9.3-dev. Further downstream images were generated with the R package Phyloseq (McMurdie et al., 2013).

2.4.6. Prediction of microbial function

Phylogenetic Investigation of Communities by Reconstruction of Unobserved Species (PICRUSt), a tool that employs the 16S rRNA gene as a marker (Langille et al., 2013) using the 13_5 version of the Greengenes database for taxonomy and OTU assignments, was used to predict the functionality of the faecal/intestinal microbiota of the low, medium and high RFI pigs. Prediction of functions was inferred based on Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations for level 3 pathways. Pathways for which the relative abundance was <0.001% were dismissed.

2.4.7. Total bacterial quantification using quantitative PCR

Quantification of the 16S rRNA gene was performed by quantitative PCR (qPCR) for all faecal and digesta samples collected in this study. A standard curve was prepared using 10-fold serial dilutions (10^9 to 10^2 copies of 16S rRNA gene/µL cloned

into the pTOPO plasmid). The plasmid was first linearized and purified and the number of copies of the plasmid determined. Reactions for standards and samples were run in triplicate on a Light Cycler 480 (Roche, Mannheim, Germany) using the following conditions: denaturation at 95 °C x 3 min, followed by 45 amplification cycles of 95 °C x 10 s, 60 °C x 20 s and 72 °C x 1 s. The reactions were performed in a final volume of 20 µL using 10 µL of Kapa SYBR fast mastermix (KapaBiosystems, London, UK) and 0.4 µM of each primer (16S rRNA forward ACTCCTACGGGAGGCAGCAG and 16S rRNA reverse ATTACCGCGGCTGCTGG), 7.2 µL of water and 1 µL of DNA. Averages were calculated and the values were then converted to number of copies/µL of total DNA extracted.

2.4.8. Volatile fatty acid analyses of faecal and intestinal digesta samples

Volatile fatty acid concentrations were measured in triplicate in faecal, ileal and caecal digesta samples. Approximately 8 g of sample was weighed and pH-recorded, diluted with 5 % TCA (2.5 × weight of sample) and centrifuged at 1,800 × *g* for 10 min at 4 °C. One and a half mL of the resultant supernatant and 1.5 mL internal standard were mixed gently, and filtered through a 0.45 µm Whatman filter (VWR International Ltd, Dublin, Ireland) into a labelled 8 mm amber GC vial (Antech Solutions Ltd., Waterford, Ireland). Extracts were stored at -80 °C until analysis. Analysis was by gas chromatography, as previously described (Prieto et al., 2014, Lynch et al., 2008).

2.4.9. Statistical analysis

Residual feed intake was calculated between weaning and day 126 pw as the residuals from a least squares multiple-regression model of ADFI on ADG, metabolic live weight, sex and all relevant two-way interactions, as well as the effects of back-fat and muscle depth, using PROC GLM in SAS 9.3. (SAS, 2011).

Growth performance parameters (weight, ADG, ADFI, FCE) were analysed using a fixed effects linear model with sex, RFI rank, time period (bi-weekly weight and feed intake recordings; repeated measure), and a two-way interaction between RFI rank and time period considered as fixed effects. Body weight at weaning (initial weight) was included as a covariate in the analysis. Sow was used as the random effect and a repeated measures model was used to describe correlations between time periods. Physiological parameters measured at only one time point (i.e. ileal histology and salivary cortisol concentrations assessed at and prior to slaughter, respectively) were analyzed using a mixed linear model also; with the aforementioned fixed effects included in the model. Body weight at slaughter was used as a covariate in the analysis of organ (liver, lungs, heart, kidney), stomach and small intestine weights and carcass cold weight as a covariate for carcass traits (muscle depth, fat and lean meat percentage). The full model was fitted using the Mixed procedure of SAS 9.3. Detailed comparisons of means were carried out using a Tukey correction for multiplicity to adjust P-values for the pairwise comparisons using t-tests. Residual checks were made to ensure that the assumptions of the analysis were met.

Statistical differences for microbiota composition between high, medium and low RFI were calculated in R using the SILVA 16S specific database (version 111), and were estimated using the Kruskal-Wallis test for independent samples and the Wilcoxon-Rank test for paired samples. Corrections for multiple comparisons were made using the Benjamini-Hochberg method. The qPCR data (following $\log_{10}$ transformation) and VFA concentrations were analysed using a generalised linear mixed model (PROC GLIMMIX) in SAS, with the same fixed effects used in the model, as described above (faeces: repeated measures analysis).

For all data, only significant differences between high and low RFI pigs are discussed and in addition for microbial composition data, only those significantly

different bacterial taxa which were present at >0.001 % median relative abundance are discussed.

Spearman rank-order correlations were performed between physiological measures found to be significantly different between low and high RFI pigs (i.e. ileal isobutyric acid concentrations and ileal goblet cell numbers) and RFI value and taxonomic relative abundances at the phylum and genus level for each sample type. Correlations were calculated using the PROC CORR procedure in SAS 9.3, and multiple comparisons were corrected for using the Stepdown Bonferroni test. A heatmap showing correlations was produced in R (Heatmap3 package).

2.4.10. Sequence data accession number

The 16S rRNA gene sequence data were deposited in the European Nucleotide Archive (ENA) under the study accession number PRJEB19324.

2.5. Results

2.5.1. Growth performance of pigs ranked by residual feed intake

The mean growth performance between weaning and day 126 pw is presented according to RFI rank in Table 2.1. Selected pigs in the low, medium and high RFI ranks had distinct RFI values, with RFI reduced by 127 g/day for pigs ranked as low compared with that of pigs ranked as high RFI ($P<0.001$). Pigs with low RFI were the most feed efficient, as indicated by a reduction in ADFI of 219 g/day ($P<0.001$), but showed an improvement in FCE of 0.12 g/g ($P<0.01$) compared to high RFI pigs. However, medium RFI pigs had statistically similar ADFI and FCE to both the high and low RFI ranks. No differences between RFI ranks were observed for ADG ($P>0.05$) or for any of the carcass quality measures (Table 2.1) or organ weights ($P>0.05$; Table S2.2).

2.5.2. Salivary cortisol and ileal histology in pigs ranked by residual feed intake

Cortisol concentrations were measured, as cortisol has been suggested as a biomarker for FE, with more feed efficient animals having lower serum concentrations (Richardson et al., 2004). However, in the present study, salivary cortisol concentrations, measured in pigs at the end of the finishing period, were unaffected by RFI rank ($P>0.05$; Table S2.2).

As intestinal structure is another factor which can potentially influence FE, ileal histology measurements were determined in the present study (i.e. villus height and width, crypt depth and villus height to crypt depth ratio, as well as the number of goblet cells; Table S3). However, only the number of goblet cells were affected by RFI rank, with low RFI pigs having 7.5 fewer goblet cells per villus and 0.02 fewer goblet cells/μm of villus height compared to high RFI pigs ($P<0.05$; Table S2.3).

2.5.3. Microbial load and diversity in pigs ranked by residual feed intake

The total bacterial load was quantified in all faecal and digesta samples. No differences were observed between high and low RFI pigs in the faeces at any time point, nor in the ileal and caecal digesta collected at slaughter (P>0.10; Table S2.4). Likewise, no significant differences for any of the indices of α-diversity measured i.e. richness based on rare OTUs (Chao1) or richness and evenness (Shannon and Simpson) were observed between RFI ranks (Figure S2.1). Furthermore, ß-diversity analyses showed that samples did not cluster based on RFI rank, but clustering on the basis of sample type was observed, with ileal samples distinctly different from faecal and caecal samples (Figure 2.2). Faecal samples also clustered according to age, with the greatest variance detected for weaning samples. No sex-associated differences were observed for intestinal microbial diversity (data not shown).

2.5.4. Intestinal microbiota composition in pigs ranked by residual feed intake

From a taxonomic perspective, 21 phyla, 161 families and 295 genera were identified across all pig faecal/intestinal samples. Phylum profiles differed depending on sample type (Figure 2.3). For example, *Firmicutes* and *Bacteroidetes* were the most abundant phyla in the faeces and caecal digesta. However, a distinct profile was observed in the ileum, where *Firmicutes* and *Actinobacteria* predominated. Other general observations at phylum level included the fact that *Proteobacteria* were more relatively abundant in the caecal and ileal digesta than in the faeces. Also, *Spirochaetaes* increased in relative abundance in the faeces as the pigs aged, and was present in the caecal, but not in the ileal digesta. However, no sex-associated differences were observed for intestinal microbial composition (data not shown).

Certain taxa were identified as differentially abundant according to RFI rank (Figure 2.3 and 2.4). Differences between low and high RFI pigs were detected for two phyla; candidate division TM7, which was 2.5-fold lower in relative abundance in the faeces of low RFI pigs at day 138 pw ($P<0.05$) and *Fusobacteria* which was 14-fold higher in the ileum of low RFI pigs ($P<0.05$); *Actinobacteria* also tended to be almost 3-fold lower in abundance in the ileum of low RFI pigs ($P=0.06$; Figure 2.3). Six bacterial families and 12 genera also differed in relative abundance between high and low RFI pigs ($P<0.05$), the details of which are outlined below, with five of the genus-level differences reflecting differences at the family level (i.e. relative abundances were identical; Figure 2.4). Relative abundance differences were observed across all sample types, but mostly in the faeces at day 138 pw (Figure 2.4C). Biological variation in microbial composition occurred between individuals, as evidenced by outliers in the relative abundance plots (Figure 2.4), although only four pigs (one low RFI and three high RFI) had outlying data for more than one taxon (but not for all taxa).

At the family level, *Erysipelotrichaceae* was 2-fold lower in the faeces collected at weaning and *Streptococcaceae* (*Streptococcus* spp.) was 1-fold lower in the faeces collected at day 42 pw, in low compared to high RFI pigs ($P<0.05$, Figure 2.4A and B). At day 138 pw (Figure 2.4C), differences occurred mainly within the *Firmicutes* phylum, and mostly for members of the *Clostridiales.* Within this order, median relative abundance of the genus *Clostridium sensu stricto1* (belonging to the *Clostridiaceae* family) was 2-fold lower, an uncultured member of the vadinBB60 family, an uncultured genus from the *Christensenellaceae* family, and the *Cellulosilyticum* genus were respectively 1-fold. 2.5-fold, and 6-fold higher in low RFI pigs than in their high RFI counterparts ($P<0.05$). Within the *Bacteroidetes* phylum, *Bacteroides* spp. was 4.5-fold higher in low versus high RFI pigs, while the genus "*candidatus* Saccharimonas" was 2-fold lower in low compared to high RFI pigs

(P<0.05, Figure 2.4C), with the latter accounting for the difference observed at the phylum level (i.e. relative abundances were identical). In the caecum, low RFI pigs had a >3-fold lower abundance of *Solobacterium* spp. but a 4.5 fold higher abundance of *Actinobacillus* spp. (P<0.05, Figure 2.4D).

In the ileum of low RFI pigs, the tendency for a lower relative abundance of *Actinobacteria* (Figure 2.3) was reflected by a concomitantly lower relative abundance (3-fold) of the *Nocardicaceae* family and the *Rhodococcus* genus (P<0.05; Figure 2.4E). Of all the taxa that differed between low and high RFI pigs, these were at the highest relative abundance. The genus *Methanosphaera* from *Archaea* was also lower in the ileum of low RFI pigs. However, low RFI pigs had a higher abundance of *Oscillibacter* spp. (from *Clostridiales*), although one pig in the low RFI rank appears to have skewed the data (P<0.05, Figure 2.4E).

The RFI-associated differences outlined above were generally mirrored at the OTU level (Figure S2.2, Table S2.5). However, there were some discrepancies; for example, at weaning and day 42 pw no differences between RFI ranks were found (Table S2.5). Furthermore, in the ileum, *Treponema berlinense* from *Spirochaetaceae* was found at higher relative abundance in low RFI pigs and in the caecum, an uncultured *Clostridiales* bacterium from *Ruminococcaceae* was present at lower relative abundance in low RFI pigs while *Actinobacillus porcinus* was higher in the low RFI pigs (Table S2.5).

Eighty percent of the OTUs in the faeces collected at weaning were common between high and low RFI pigs, 85% were common at day 42 pw, and 82% at day 138 pw and in the caecal digesta and 66% in the ileal digesta. On the other hand, a number of OTUs (belonging to 17 phyla) were found exclusively in either low or high RFI ranked pigs (Figure S2.2). Low RFI pigs harboured more of these OTUs compared to high RFI pigs, in particular in the ileum, where 60 OTUs were found to be exclusive to

low RFI pigs versus 28 in their high RFI counterparts. Some of the OTUs exclusively found in low RFI pigs represent potentially beneficial microbes; for example *Akkermansia* found in the faeces at weaning and in the ileum, *Bifidobacterium* in the faeces at day 138 pw, uncultured bacteria from *Prevotellaceae* in the faeces at weaning and day 138 pw, *Mucispirillum* in the faeces at day 42 pw and in the caecum, and *Butyricimonas* in the ileum. Most of the RFI-specific OTUs were members of *Firmicutes*, especially the uncultured microorganisms and those from the *Clostridiales* order.

2.5.5. Intestinal microbiota correlations with RFI value

A correlation analysis was performed between the intestinal microbiota composition, at the phylum and genus levels, and RFI value, and although no significant RFI-associated correlations were found at the phylum level, 13 genera correlated with RFI value ($P<0.05$; Figure 2.5); eight with low RFI (negative correlation) and five with high RFI (positive correlation) across the different sample types. An uncultured organism of the vadinBB60 family, was correlated with a low RFI value i.e. with better FE, in the faeces at day 138 pw ($P<0.05$), and was the only RFI-correlated genus previously identified as RFI-associated from the relative abundance data (Figure 2.4C). *Butyrivibrio*, from which two uncultured OTUs were found exclusively in the faeces of low RFI pigs at day 42 pw, was strongly correlated with a low RFI value at weaning ($P<0.05$). Furthermore, *Prevotella*, in the faeces collected at weaning, *Corynebacterium* and *Defluviitaleaceae* Incertae Sedis in the faeces collected prior to slaughter (day 138 pw), *Lactobacillus* in the caecal digesta, and *Brevibacterium* and *Anaeroplasma* in the ileal digesta, were also correlated with a low RFI value, although not differing in relative abundance between high and low RFI pigs. On the contrary, *Anaeroplasma*

was strongly correlated with a high RFI value in the faeces on day 42 pw and other weaker correlations were found with microbes in the caecum and ileum.

2.5.6. Predictive functional analysis of the intestinal microbiota of pigs ranked by residual feed intake

PICRUSt was employed in order to gain some insight into the functional capacity of the intestinal microbiota (Langille et al., 2013) of the pigs in the present study in order to explore any potential links with FE. Between 47 and 93% of the sequences were taxonomically assigned in the Greengenes database with 97% homology. Most of the predicted pathways identified were at very low median relative abundance (0.001% to 0.99%; Figure 2.6). Nine predicted microbial pathways differed significantly in abundance between low and high RFI pigs; two in the faeces at day 138 pw and one in the caecal digesta, but most (i.e. six) were in the ileal digesta, where another two pathways also tended to be different ($P \leq 0.10$). The differentially abundant predicted pathways at highest relative abundance were mostly related to metabolic function in the ileum of low RFI pigs. These were enriched in low RFI pigs and included pathways involved in the biosynthesis of amino acids (phenylalanine, tyrosine, tryptophan, valine, leucine and isoleucine) and metabolism of C_5-branched dibasic acid, terpenoids and polyketides as well as restriction enzyme processing. Higher restriction enzyme activity in the faecal microbiota of low RFI pigs on day 138 pw was also inferred. Furthermore, translation factors tended to be higher in relative abundance in low compared to high RFI pigs ($P=0.06$). Contrary to this, some of the bacterial pathways inferred were at lower relative abundance in low RFI pigs; for example those involved in the biosynthesis of secondary metabolites in the faeces at day 138 pw, thiamine metabolism in the caecum, and the phosphotransferase system (PTS) in the

ileum. A pathway involved in bacterial invasion of epithelial cells also tended to be less abundant in the ileum of low RFI pigs (P=0.08).

2.5.7. Volatile fatty acid concentrations in the faeces and digesta of pigs ranked by residual feed intake

Volatile fatty acid concentrations measured in the faeces collected throughout the lifetime of the pigs, and in the caecal and ileal digesta collected at slaughter are presented in Tables S2.6 and S2.7. Only one difference was found between low and high RFI pigs; low RFI pigs had a 2.3-fold higher concentration of isobutyric acid in the ileal digesta compared to high RFI pigs (P<0.05; Table S2.7).

2.5.8. Correlations between microbial composition and physiological traits in pigs ranked by residual feed intake

Correlations were examined between bacterial taxa (at both the phylum and genus levels) and those physiological measures found to differ significantly between low and high RFI pigs (Figure 2.5). In the ileum, the concentration of isobutyric acid was positively correlated with the relative abundance of *Asteroleplasma*. The number of goblet cells in the ileum (both per villus and per µm villus height) was negatively correlated with *Proteobacteria* and an uncultured bacterium from *Christensenellaceae* (P<0.05). The phylum *Synergistetes*, an uncultured genus from *Veillonellaceae*, as well as the genera *Cellulosilyticum* and *Parvimonas* were positively correlated with goblet cell number per µm villus height (P<0.05). Of these, both the uncultured member of *Veillonellaceae* and the *Cellulosilyticum* genus were also positively correlated with the number of goblet cells per villus (P<0.05).

2.6. Discussion

The advent of high-throughput sequencing has facilitated comprehensive profiling of the resident bacteria in the digestive tract of pigs (Buzoianu et al., 2012b, Mach et al., 2015, Buzoianu et al., 2013, Kim et al., 2015b). However, this study is one of the first to exploit this technology to examine the intestinal microbiota among pigs of varying FE. The metric used for FE was RFI, and ranking pigs according to this measure was particularly useful as it allowed the selection of pigs that consume less feed to achieve the same weight gain, in agreement with previous findings for pigs divergent in RFI (Vigors et al., 2016a, Patience et al., 2015). In order to minimize the variability in FE due to external factors, pigs were ranked on RFI within litter (to control for genetic influences) and all pigs were subjected to the same management, environmental, and nutritional conditions. When considering reasons why pigs may differ in FE, it is interesting to note that the energy-related physiological parameters measured in the present study were not associated with RFI. For example, there was no difference in stress levels, as determined by salivary cortisol concentrations, despite serum cortisol having previously been suggested as a biomarker for FE in cattle (Richardson et al., 2004). Furthermore, low RFI pigs tended to have higher plasma cortisol concentrations in a previous study (Lefaucheur et al., 2011), albeit no differences in salivary concentrations were found, in agreement with our findings. In addition, there were no differences in carcass weight, leanness or organ weights among animals divergent in RFI in the present study, although previous work has found that low RFI pigs have lower back fat (Cai et al., 2008). However, there are likely unmeasured attributes contributing to FE.

When looking at the intestinal microbiota, similar to previous findings in cattle, the overall intestinal bacterial diversity did not cluster by RFI rank, but rather RFI-

associated variations in community membership were detected (Myer et al., 2015, Jewell et al., 2015). Interestingly, in our study, microbial diversity as well as composition, was impacted by both the age and intestinal site. Furthermore, in faeces taken at three time points, clusters converged with age, indicating that the intestinal microbiota became more homogenous among pigs over time. In agreement with previous studies (Xiao et al., 2016, Zhao et al., 2015, Mach et al., 2015), the core phyla within the faecal and caecal microbiota were *Firmicutes* and *Bacteroidetes*. However, the ileal microbiota composition differed to that previously found in pigs (Yang et al., 2016), with *Actinobacteria* replacing *Bacteroidetes* as the second most abundant phylum.

The hypothesis that the composition and potential functionality of the intestinal microbiota is linked with FE in pigs was supported by the differences in faecal/intestinal bacterial profiles found between RFI ranks throughout the lifetime of pigs. However, these differences can be considered subtle, as of all the taxa detected, relatively few differed and most that did were present at low relative abundance (<2%). Nonetheless, these taxa may still influence FE, as ultimately, it is the complex interplay within the intestinal community that would have the most influence on host homeostasis and FE. On the other hand, we cannot disregard the fact that biological variation in microbial composition between pigs could account for some of the differences found. It may also be that FE is influencing the intestinal microbiota, meaning that pigs with low RFI are more feed efficient for a number of reasons and because of this they have a somewhat different microbiota; but further studies are needed to elucidate such causality. Furthermore, as sex influences FE in pigs, one might expect differences in the microbiota profile due to sex, as previously reported (Zhou et al., 2015). However, no association with sex was observed in the present study.

As outlined above, most of the differences in the composition and predicted functionality of the faecal and intestinal microbiota observed between RFI ranks were subtle. Several members of the *Clostridiales* order previously associated with carbohydrate degradation and better metabolic efficiency (i.e. a leaner phenotype in humans and less fatness in pigs) were enriched in low RFI pigs in the faeces immediately pre-slaughter e.g. uncultured *Christensenellaceae* and *Cellulosilyticum* (Yang et al., 2016, Miller et al., 2011, Goodrich et al., 2014, Mao et al., 2015). Additionally, OTUs exclusively found in low RFI (more feed efficient) pigs are from this order, including those that were higher in abundance in these pigs; for example, an unknown genus belonging to the vadinBB60 family. Previously, an unclassified genus belonging to this family was increased in relative abundance in rats fed a high-fat diet for 4 weeks i.e. during the pre-obese state, indicating its possible role in metabolism (Lin et al., 2016).

At weaning, the butyrate producer, *Butyrivibrio*, was strongly correlated with low RFI which could also be linked to an enhanced ability to ferment complex carbohydrates (Hespell et al., 1987). Moreover, *Prevotella*, a member of *Bacteroidetes* considered another key microbe capable of fermenting complex carbohydrates (Umu et al., 2015, Flint et al., 2008b), was correlated with a low RFI value at weaning. The fact that this was observed at weaning, may be due to the introduction of a cereal-based diet and its likely role in enhancing growth rate post-weaning (Mach et al., 2015). These findings indicate that more feed efficient pigs are likely to have an intestinal microbiota that is more competent in terms of digesting the carbohydrate component of the diet. Some of these microbes could also have a role in influencing ileal morphology as indicated by a negative correlation between an uncultured *Christensenellaceae* OTU (enriched in low RFI pigs) and the number of goblet cells per µm of villus height (lower in low RFI pigs). Furthermore, increases in members of *Bacteroidetes* have been

reported as a driver for leaner phenotypes (Yang et al., 2016, Turnbaugh et al., 2009, Torok et al., 2011, Wu et al., 2011), which was substantiated in the present study by an enrichment of *Bacteroides* spp. in low RFI pigs. On the contrary, some other members that may have a role in carbohydrate utilisation, such as the genus *Candidatus Saccharimonas* (Niu et al., 2015) or *Methanosphaera*, were less abundant in low RFI pigs, albeit they were present at very low median relative abundance. *Actinobacillus* spp. was present at a higher abundance in the caecum of low RFI pigs, but despite this, some species are potentially pathogenic for pigs (OTUs of *A. pleuropneumoniae, A. porcitonsillarum* and *A. rossii*) which were found, albeit at very low abundance, in the ileum of both low and high RFI pigs. Potentially undesirable bacteria that were less abundant in more feed efficient pigs included the *Erysipelotrichaceae* family, associated with intestinal inflammation in humans (Kaakoush, 2015). In addition, genera with a possible negative effect on FE, as they contain potentially pathogenic members e.g. *Streptococcus* and *Solobacterium* (Goyette-Desjardins et al., 2014, Pedersen et al., 2011), were less relatively abundant in more feed efficient pigs in the faeces at day 42 pw and in the caecum at slaughter, respectively, albeit they were at low relative abundance in all groups.

The ileal microbiota was notable by virtue of the number of unique FE-associated OTUs harboured, and by the fact that the bacterial metabolite isobutyric acid was found at a higher concentration in the ileum of more feed efficient pigs, albeit at a relatively low concentration in both groups. Isobutyric acid is an end-product of protein fermentation, and increased concentrations could be indicative of better utilisation of dietary protein by the microbiota (Walsh et al., 2013). Furthermore, the predicted higher relative abundance of the valine, leucine and isoleucine biosynthesis pathway in low RFI pigs may be linked to the higher concentration of isobutyric acid in the ileum of these animals, as isobutyric acid is the end product of microbial deamination of

valine (Zarling et al., 1987). On the other hand, it could also mean that these pigs are less efficient at digesting protein, leaving more available for microbial fermentation. This is especially noteworthy as it occurred in the ileum. However, poorer protein utilization would not be expected in more feed efficient pigs. As regards the ileal microbiota, the most notable difference in relative abundance across RFI-ranked pigs occurred for *Rhodococcus* spp., a genus containing species known to cause disease in pigs [specifically infections of the submaxillar and mesenteric lymph nodes (Lara et al., 2011, Komijn et al., 2007)], which was substantially lower in the low RFI pigs. This provides evidence that the microbiota of more feed efficient pigs could potentially be "healthier". In addition, the high relative abundance of this genus is remarkable, as, to our knowledge, it has not previously been reported as abundant in pigs.

In addition, ileal histology is important when considering FE. For example, longer villi and shorter crypts enhance absorptive capacity (Chen et al., 2014, Pluske, 2002, Lalles, 2007). However, while we found no differences in these parameters between pigs of varying FE, we did find fewer goblet cells along the villi of more feed efficient pigs. This suggests reduced mucin secretion in these animals, perhaps indicating increased nutrient absorptive capacity, as excess mucin can act as a physical barrier to absorption (Montagne et al., 2004). It may also indicate less diversion of energy away from growth, as the animals are producing less mucin. Both of these hypotheses may help to explain the better FE in these animals. In agreement with the potentially lower mucin production in low RFI pigs, the *Clostridium sensu stricto 1* genus, less abundant in the faeces of these animals, is a mucin promoter (Wlodarska et al., 2015), while *Mucispirillum*, an opportunistic mucin degrader, previously found to play a role in active colitis in murine models, was more abundant in the caecum (Rooks et al., 2014). Within the *Clostridiales* order, butyric acid-producing bacteria, and mucin degraders, for which a greater number of OTUs were found in low RFI pigs, have been

associated with improved gastrointestinal health in humans and other animals, including pigs (Berni Canani et al., 2016, Levine et al., 2013), likely through increased mucin production in the colon, which enhances epithelial barrier function (Burger-van Paassen et al., 2009). It is noteworthy that *Akkermansia*, among other OTUs exclusively found in the ileum of more feed efficient pigs, is also linked with mucin degradation, and can indicate better/healthier intestinal function, as it has been inversely correlated with metabolic disorders and intestinal inflammation (Schneeberger et al., 2015). The involvement of different metabolic pathways, predicted to be either more or less relatively abundant in more feed efficient pigs, could further justify differences in the host phenotype. For instance, genes encoding the PTS, a bacterial sugar transport system, were predicted to be more relatively abundant in the small intestine of pigs with poor FE. This could be linked with a higher bacterial energy uptake, leaving less sugar available for growth of the animal (Yang et al., 2016, Goodrich et al., 2014, Deutscher et al., 2006).

2.7. Conclusion

In conclusion, this study has revealed FE-related compositional differences within the intestinal microbiota throughout the life of the pig, but mostly at the end of the finishing period, suggesting that the intestinal microbiota has a possible link with FE in pigs. Specifically, a higher relative abundance of potentially beneficial bacteria, most notably members of *Clostridiales* and *Bacteroidetes*, and a lower relative abundance of potentially undesirable bacteria, such as *Rhodococcus*, and *Erysipelotrichaceae*, were found in more feed efficient animals. However, it should be borne in mind that many of the FE-associated compositional differences were relatively subtle, occurring for taxa present at low relative abundance. Nonetheless, the differentially abundant intestinal taxa identified could potentially be exploited as biomarkers for FE or manipulated by dietary means in order to improve FE. Although, when examined at the genus and OTU level, some members of these taxa were uncultured, advances in culturing techniques may facilitate their exploitation in the future. However, additional research is needed in order to investigate the reliability of the FE-associated microbial taxa identified here i.e. across batches of pigs/rearing environments. Furthermore, intervention studies are required in order to confirm the insights provided so as to improve FE in pigs.

2.8. Tables and Figures

Table 2.1. Effect of ranking pigs on residual feed intake (between weaning and day 126 post-weaning) on growth performance parameters and carcass traits

Parameter[1]	High RFI	Medium RFI	Low RFI	S.E.M[2]	P-value
RFI (g/day)	76.0[a]	6.0[b]	-51.0[c]	15.40	<0.001
ADG (g/day)	910	877	855	28.4	0.38
ADFI (g/day)	1850[a]	1732[a,b]	1631[b]	51.2	<0.01
FCE (g/g)	1.91[a]	1.86[a,b]	1.79[b]	0.025	<0.01
Slaughter weight (Kg)	150.3	147.2	141.0	2.50	0.51
Carcass cold weight (Kg)	113.4	113.1	108.1	3.59	0.48
Kill out (%)	79.2	78.9	77.9	0.53	0.19
Muscle depth (mm)	61.7	61.0	63.2	1.78	0.66
Fat (mm)	17.2	17.9	16.4	0.79	0.56
Lean meat (%)	54.9	54.1	55.5	0.69	0.49

High RFI (n=10), medium RFI (n=10), low RFI (n=12).

[1]RFI: Residual Feed Intake, ADG: Average Daily Gain; ADFI: Average Daily Feed Intake; FCE: Feed Conversion Efficiency.

[2]Least squares means and pooled standard errors of the means are presented.

[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P \leq 0.05$).

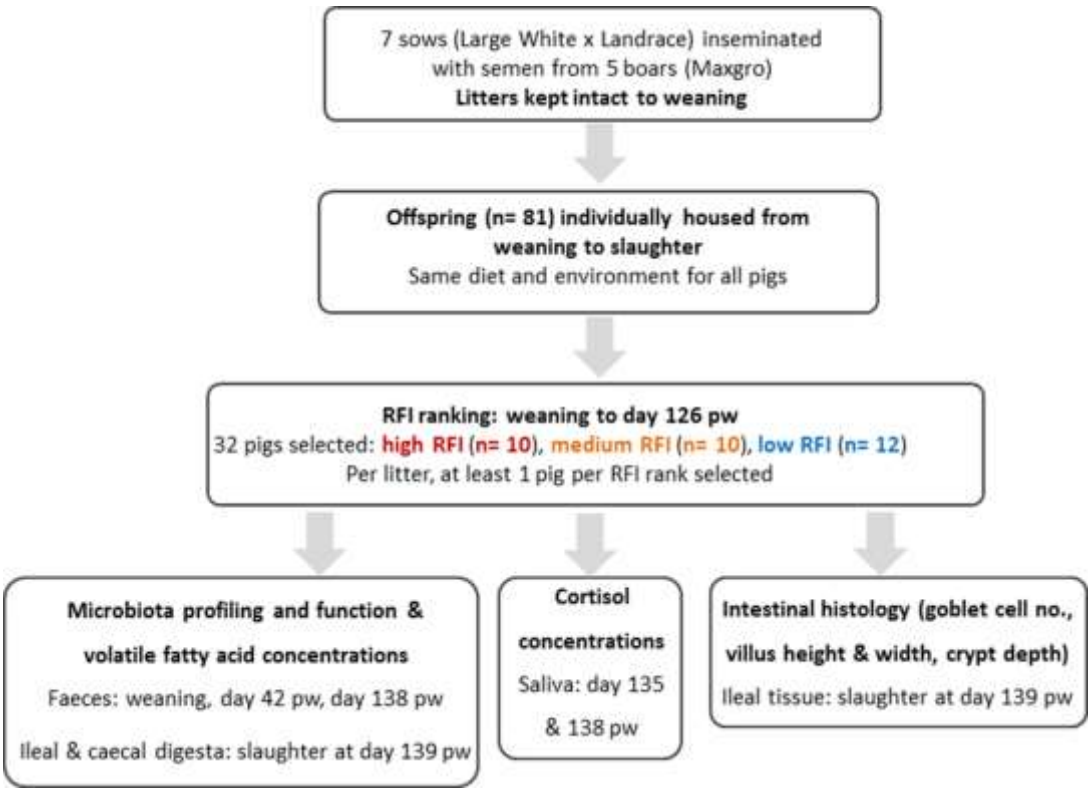


Figure 2.1. Flow chart depicting animal management, selection and sample collection
[1]RFI: residual feed intake; [2]pw: post-weaning

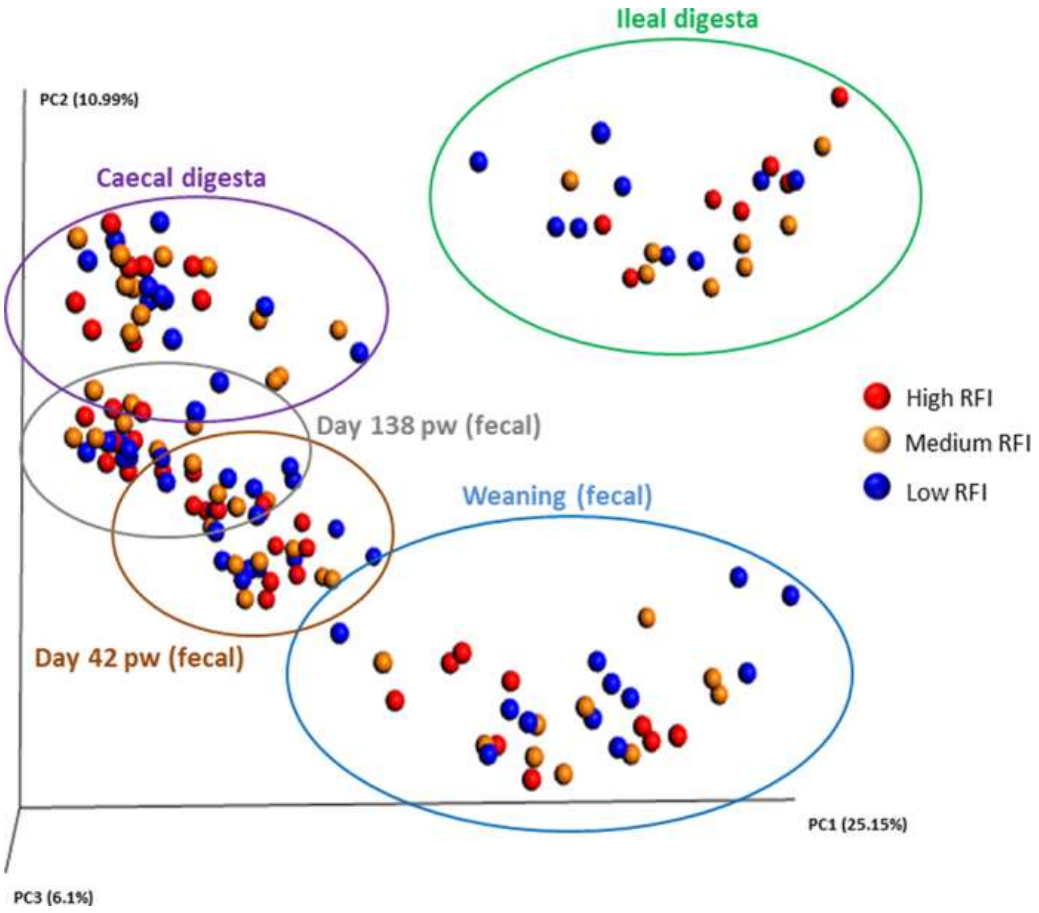


Figure 2.2. Principal coordinate analysis (PCoA) plot (based on OTUs) according to residual feed intake (RFI) rank and sample type (n=150)

Low RFI: faeces (n=36), digesta (caecal n=12, ileal n=9); medium RFI: faeces (n=30), digesta (caecal n=10, ileal n=9); high RFI: faeces (n=30), digesta (caecal n=8, ileal n=6). Plot is based on the unweighted UniFrac distances. The amount of variance is depicted by the percentages in parentheses on each axis. Ellipses denote clustering according to faecal sample time points and intestinal location.

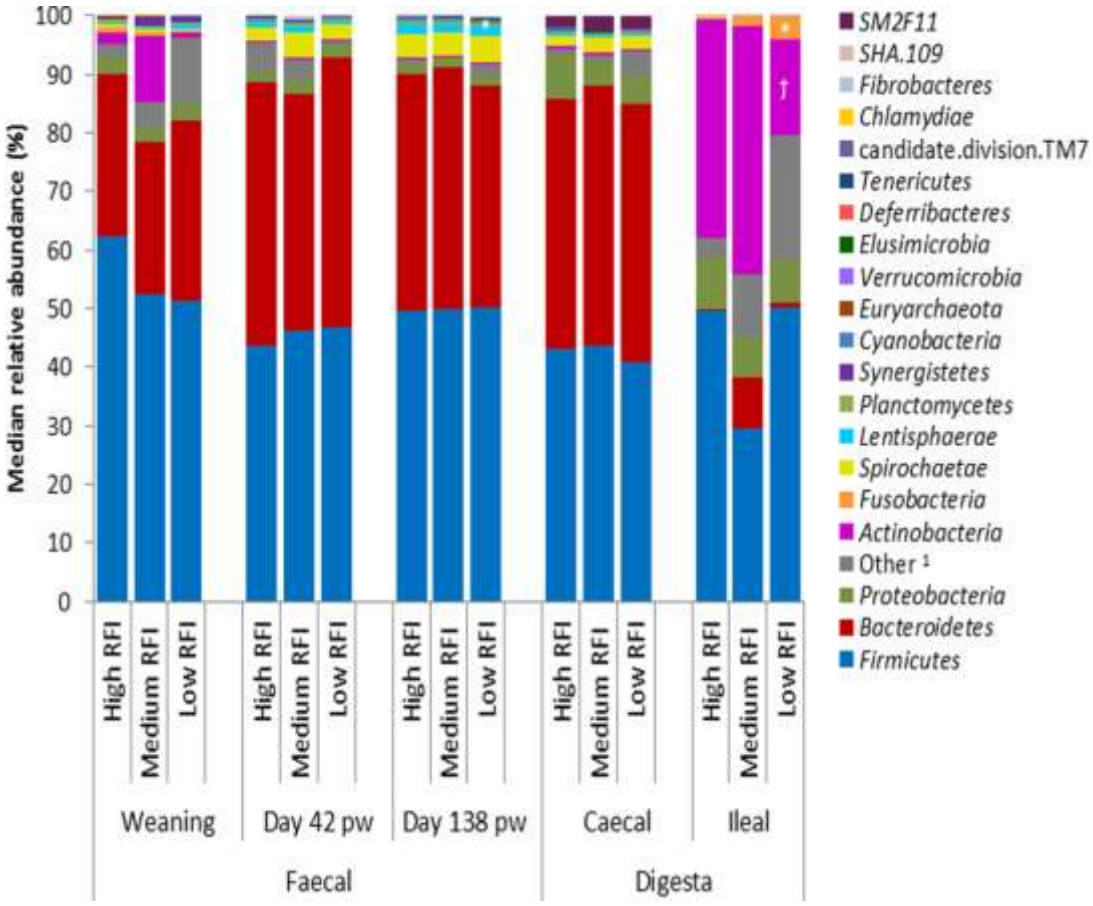


Figure 2.3. Median relative abundance (%) of bacterial phyla present in pigs ranked by residual feed intake (RFI) across all faecal time points (n=96) and both intestinal locations (n=54)

[1]No blast hits/uncultured; [*]Indicates significant differences (*Candidate Division TM7* in the faeces at day 138 post-weaning and *Fusobacteria* in the ileum; P≤0.05) and † indicates tendencies towards significant differences (*Actinobacteria* in the ileum; P=0.06) observed between high and low RFI pigs within each sample type.

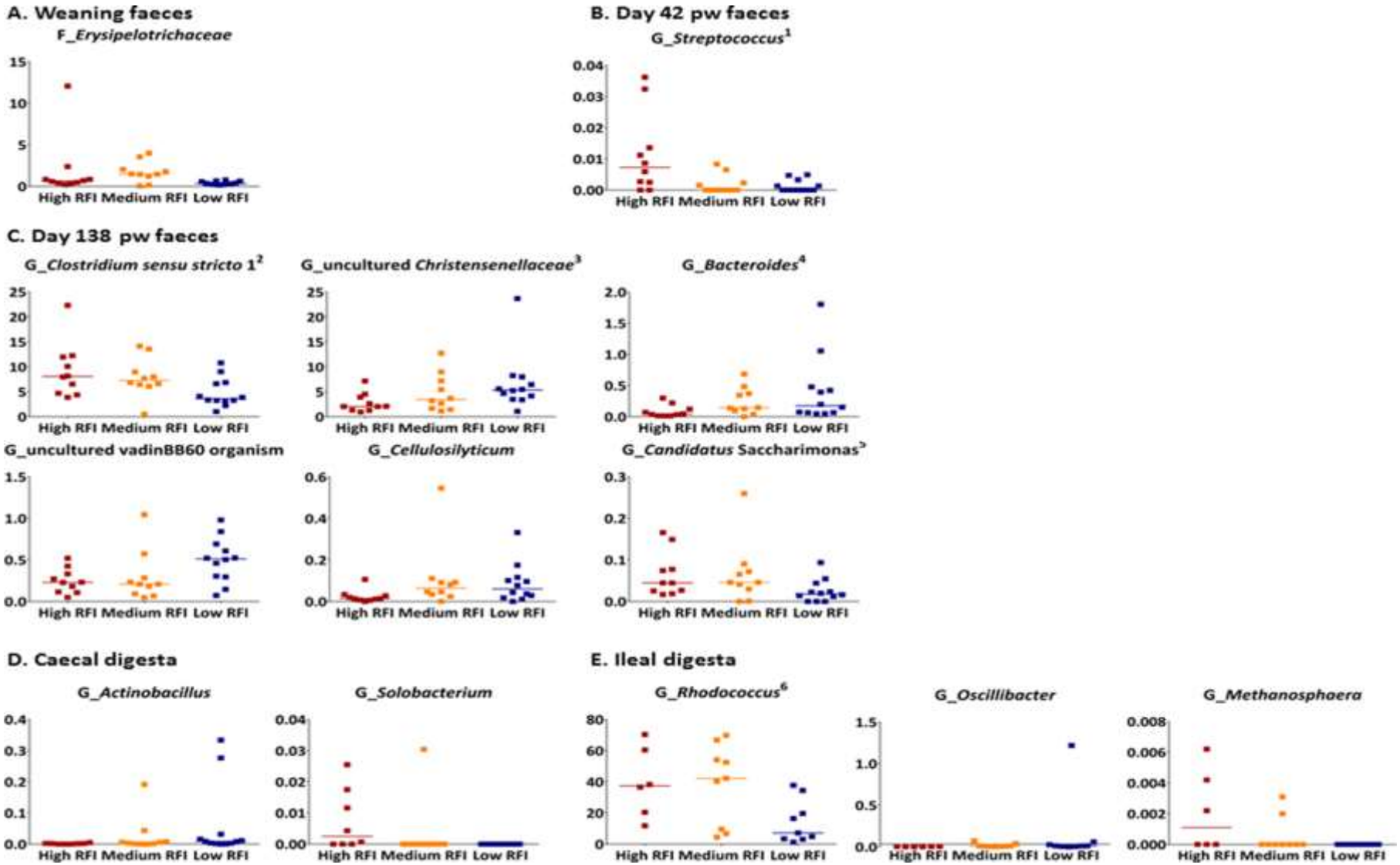

A. Weaning faeces
F_Erysipelotrichaceae
B. Day 42 pw faeces
G_Streptococcus1
C. Day 138 pw faeces
G_Clostridium sensu stricto 12
G_uncultured Christensenellaceae3
G_Bacteroides4
G_uncultured vadinBB60 organism
G_Cellulosilyticum
G_Candidatus Saccharimonas5
D. Caecal digesta
G_Actinobacillus
G_Solobacterium
E. Ileal digesta
G_Rhodococcus6
G_Oscillibacter
G_Methanosphaera
High RFI Medium RFI Low RFI

Figure 2.4. Median relative abundance (%) of microbial taxa found to be differentially abundant between pigs ranked on low and high residual feed intake (RFI) (P<0.05) in A. faeces at weaning (n=32), B. faeces at day 42 pw (n=32), C. faeces at day 138 pw (n=32), D. caecal digesta (n=30) and E. ileal digesta (n=24)

Low RFI: faeces (n=36), digesta (caecal n=12, ileal n=9); medium RFI: faeces (n=30), digesta (caecal n=10, ileal n=9); high RFI: faeces (n=30), digesta (caecal n=8, ileal n=6). F_: family; G_: genus. Horizontal lines in the plots indicate median values of the distribution. The *Fusobacteria* phylum was also differentially abundant and this is illustrated in Figure 3. Some genus-level differences shown in the plots reflect differences at a higher taxonomic level which are not shown here as follows. [1]*Streptococcaceae* family, [2]*Clostridiaceae1* family, [3]*Christensenellaceae* family, [4]*Bacteroidaceae* family, [5]Candidate division TM7 phylum and [6]*Nocardiaceae* family. Some of the animals for which the highest variance from the median values of the taxa distribution was seen had outlying data for more than one taxon i.e. one low RFI pig had a higher relative abundance than the median value for uncultured *Christensenellaceae*, *Actinobacillus* and *Oscillibacter* and three high RFI pigs had a higher relative abundance than the median value for *Rhodococcus* and *Methanosphaera*.

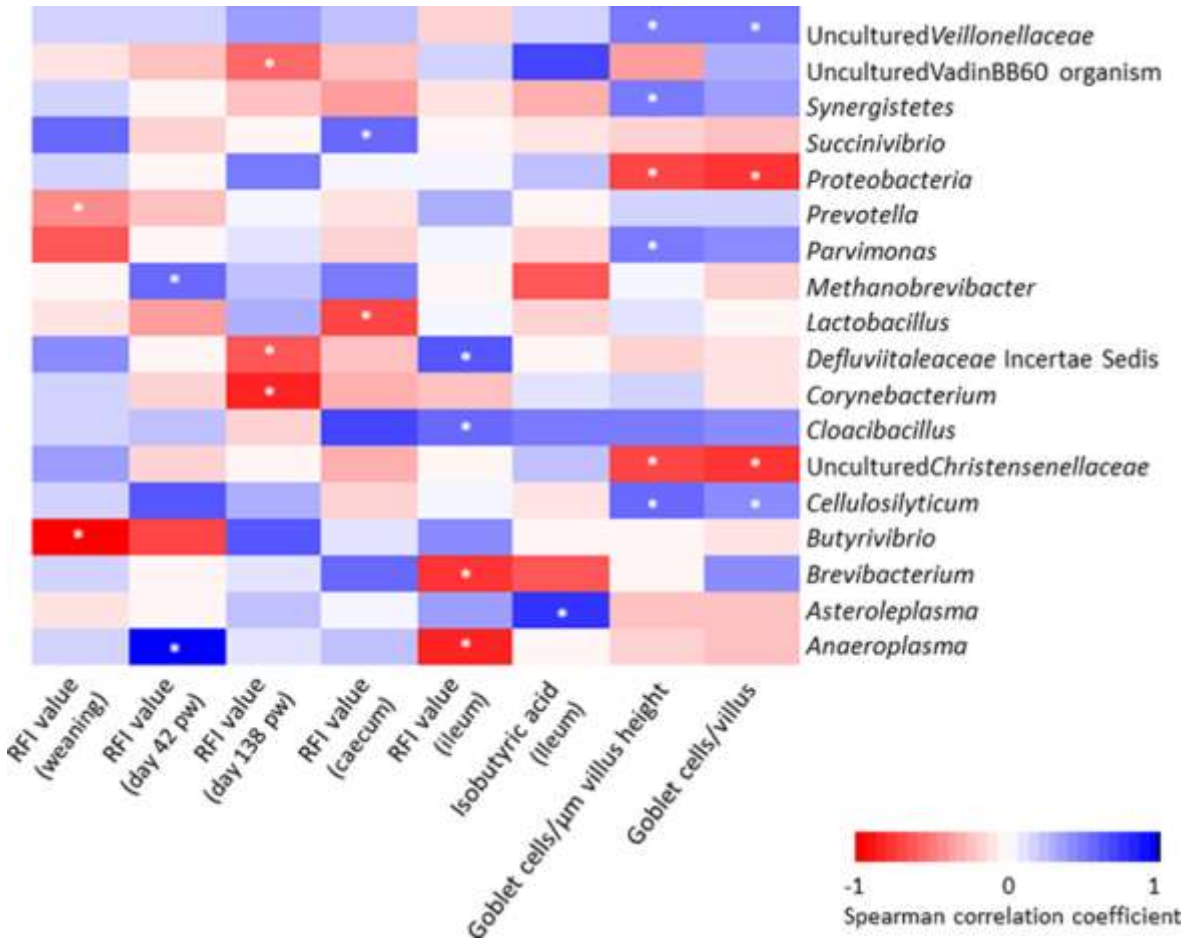


Figure 2.5. Heat map showing Spearman correlations between bacterial taxa and physiological measures in pigs ranked by residual feed intake (RFI)

Low RFI: faeces (n=36), digesta (caecal n=12, ileal n=9); medium RFI: faeces (n=30), digesta (caecal n=10, ileal n=9); high RFI: faeces (n=30), digesta (caecal n=8, ileal n=6). [1]pw: post-weaning. Correlations were examined between bacterial taxa (at both the phylum and genus levels) and those physiological measures found to be significantly different between low and high RFI pigs. *Denotes significant correlation ($P \leq 0.05$).

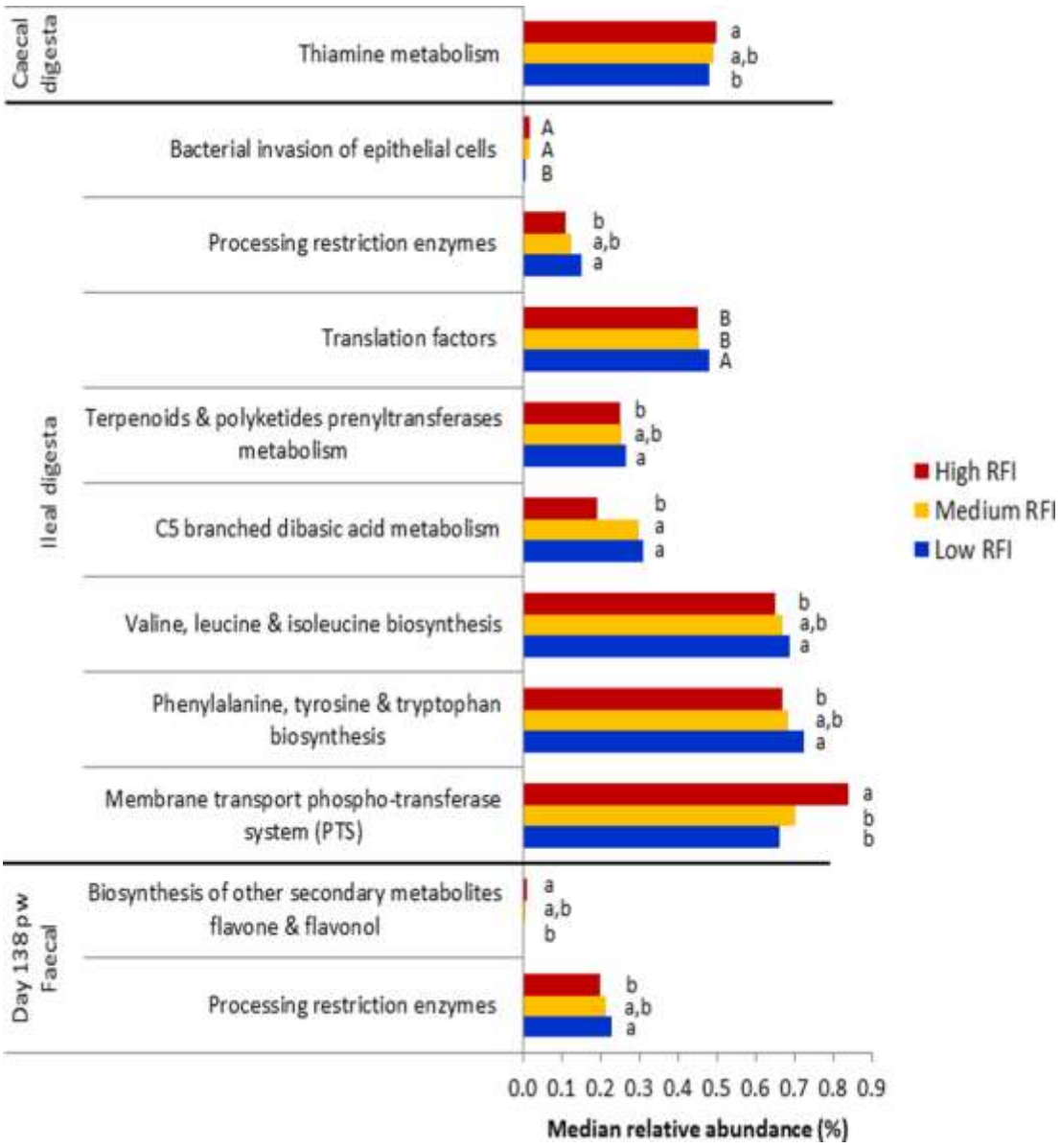


Figure 2.6. Comparison of predicted functional pathways for the faecal and intestinal microbiota of pigs ranked by residual feed intake (RFI)

Low RFI: faeces (n=12), digesta (caecal n=12, ileal n=9); medium RFI: faeces (n=10), digesta (caecal n=10, ileal n=9); high RFI: faeces (n=10), digesta (caecal n=8, ileal n=6). Pathways are from the KEGG database and level 3 pathways are presented. Only 11 of 23 differences between all RFI ranks are shown in the graphic, as 6 were pathways present at <0.001% median relative abundance and another 6 were differences observed for medium RFI pigs. [a,b,c] Within each predicted pathway, bars that do not share lowercase letter (a,b,c) are significantly different ($P \leq 0.05$), whereas those that do not share uppercase letters (A,B,C) tended to be different ($P \leq 0.10$).

2.9. Supplementary Information

Table S2.1. Composition and chemical analysis of diets used in the study (on an as-fed basis; g/kg)

Diet Type	Starter	Link	Weaner	Finisher	Pregnant sow	Lactating sow
Barley			248.0	385.4	897.4	349.5
Wheat	220.0	399.0	431.4	404.0		432.4
Maize	80.0					
Soya	163.5	229.2	200.0	175.0	70.0	150.0
Full fat soya	100.0	70.0	50.0			
Lactofeed 70[1]	200.0	200.0				
Skim milk powder	125.0	50.0				
Soya oil	78.1	25.0	40.0	10.0	10.0	40.0
Lysine HCl (78.8)	4.73	3.70	4.6	4.0	1.0	3.5
DL-Methionine	3.22	2.33	1.7	1.0		1.0
L-Threonine (98)	2.41	1.62	2.0	1.5		1.0
L-Tryptophan	0.95	0.54	0.2	0.0		
Vitamin and mineral mix	3.0[2]	3.0[2]	3.0[2]	1.0[3]	1.5[4]	1.5[4]
Natuphos 5000 FTU/g[5]	0.10	0.10	0.1	0.1	0.1	0.1
Salt feed grade	3.00	3.00	3.0	3.0	4.0	4.0
Dicalcium phosphate	5.00	1.52	5.0	2.0	5.0	5.0
Limestone flour	11.00	11.0	11.0	13.0	11.0	12.0
Chemical analysis (g/kg dry matter)						
Crude protein	235.9	252.8	211.0	205.4	195.7	172.1
Crude fibre	19.8	23.4	34.8	37.5	38.9	31.7
Crude ash	66.2	63.5	48.3	45.4	44.6	49.8
Ether extract	114.7	57.9	70.7	27.2	33.2	63.4
Digestible energy (MJ/kg)[6]	17.9	17.0	16.9	16.0	15.9	16.3
Net energy (MJ/kg)[6]	11.4	10.3	10.6	9.8	9.5	10.5
Amino acids (g/kg)						
Lysine	16.2	15.0	13.0	11.1	6.4	9.9
Methionine	6.8	5.7	4.5	3.6	2.1	3.4
Methionine + cysteine	9.7	9.0	7.9	6.8	4.7	6.4
Threonine	10.5	9.8	8.7	7.5	4.5	6.5
Tryptophan	3.6	3.3	2.6	2.2	1.6	2.0

[1]Lactofeed 70 contains 70% lactose, 11.5% protein, 0.5% oil, 7.5% ash and 0.5% fibre (Volac, Cambridge, UK).

[2]Premix provided per kg of complete diet: Cu, 155 mg; Fe, 90 mg; Mn, 47 mg; Zn, 120 mg, I, 0.6 mg; Se, 0.3 mg; vitamin A, 6000 IU; vitamin D_3, 1000 IU; vitamin E, 100 IU; vitamin K, 4 mg; vitamin B_{12}, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B_1, 2 mg; vitamin B_6, 3 mg; Endox, 60 g.

[3]Premix provided per kg of complete diet: Cu, 15 mg; Fe, 24 mg; Mn, 31 mg; Zn, 80 mg, I, 0.3 mg; Se, 0.2 mg; vitamin A, 2000 IU; vitamin D_3, 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin B_{12}, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B_1, 2 mg; vitamin B_6, 3 mg.

[4] Premix provided per kg of complete diet: Cu, 15 mg; Fe, 70 mg; Mn, 62 mg; Zn, 80 mg, I, 0.6 mg; Se, 0.2 mg; vitamin A, 1000 IU; vitamin D_3, 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; vitamin B_{12}, 15 µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; Biotin, 200 mg; Folic acid, 5 g; vitamin B_1, 2 mg; vitamin B_6, 3 mg.

[5]Phytase; 5000 FTU/g equal to 500 FTU per kg finished feed.

[6]Digestible energy and net energy were calculated from book values.

Table S2.2. Effect of ranking pigs on residual feed intake (between weaning and day 126 post-weaning) on salivary cortisol concentrations[1] and organ weights

Measure	High RFI	Medium RFI	Low RFI	S.E.M[2]	P
Salivary cortisol (ng/ml)	4.8	4.1	4.5	1.38	0.74
Heart (g)	476.5^{A}	522.2^{B}	$482.3^{A,B}$	15.54	0.08
Kidneys (g)	426.9	514.5	482.9	34.40	0.26
Liver (g)	2001.7	2054.4	2105.6	66.14	0.55
Lungs (g)	1152.6	1236.2	1130.7	56.01	0.38
Stomach (g)	595.3	615.0	661.1	26.85	0.22
Small intestine (g)	1453.5	1631.1	1595.7	112.73	0.54

High RFI: n=10, medium RFI: n=10, low RFI: n=12.

[1]Mean of data from days 135 and 138 post-weaning; [2]Least square means and pooled standard error of means are presented. ^{A,B,C} Within each row, values that do not share a common superscript tend to be different ($P \leq 0.10$).

Table S2.3. Effect of ranking pigs on residual feed intake (between weaning and day 126 post-weaning) on ileal morphology[1]

Measure	High RFI	Medium RFI	Low RFI	S.E.M[2]	P
Villus height (μm)	302.2	298.5	302.7	18.26	0.98
Villus width (μm)	116.8	117.6	153.3	15.89	0.18
Crypt depth (μm)	283.1	308.9	287.8	36.55	0.88
Villus height : crypt depth	1.13	0.94	1.37	0.16	0.18
No. of goblet cells/villus	19.6[a]	12.8[b]	12.1[b]	1.81	0.01
No. of goblet cells/μm villus height	0.06[a]	0.04[b]	0.04[b]	0.006	0.02

High RFI: n=10), medium RFI: n=10, low RFI: n=12.

[1]Ten villi were measured for each pig and the means were used for statistical analysis.

[2]Least square means and pooled standard error of means are presented.

[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P \leq 0.05$).

Table S2.4. Effect of ranking pigs on residual feed intake (between weaning and day 126 post-weaning) on total bacterial number (log_{10} copies/ng total DNA) in faecal and intestinal samples

Time point	High RFI	Medium RFI	Low RFI	S.E.M[1]	P
Faeces weaning	5.82	5.81	5.81	0.062	0.99
Faeces day 42 pw	6.22	6.18	6.19	0.066	0.91
Faeces day 138 pw	6.14	6.23	6.18	0.061	0.67
Ileal digesta	5.06	5.14	5.27	0.158	0.66
Caecal digesta	5.99	5.79	5.75	0.163	0.18

Low RFI: faeces (n=36), digesta (caecal n=12, ileal n=9); medium RFI: faeces (n=30), digesta (caecal n=10, ileal n=9); high RFI: faeces (n=30), digesta (caecal n=8, ileal n=6).

[1]Least square means and pooled standard error of means are presented.

[A,B,C] Within each row, values that do not share a common superscript tend to be different ($P \leq 0.10$).

Table S2.5. Relative abundance of OTUs statistically different[1] among RFI ranks

Sample type	OTU Taxonomy	Median relative abundance [confidence interval][2]			P-value[3]		
		High RFI (n=10)	Medium RFI (n=10)	Low RFI (n=12)	High vs Low	High vs Medium	Low vs Medium
Faeces weaning	*Firmicutes;Clostridia;Clostridiales;Family.XIII; Mogibacterium;uncultured.bacterium*	0.0 [0.0;1e-05]	3e-05 [1e-05;8e-05]	0.0 [0.0;0.0]	0.53	0.06	0.04
	Firmicutes;Bacilli;Lactobacillales;Streptococcaceae; Streptococcus.;uncultured.bacterium	1e-05 [0.0;1e-04]	0.0 [0.0;0.0]	4e-05 [2e-05;6e-05]	0.46	0.16	0.02
	Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae; dgA.11.gut.group;uncultured.bacterium	0.00133 [0.00084;0.00397]	0.00018 [0.00005;0.00035]	0.00145 [0.00045;0.00448]	0.79	0.03	0.03
	Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae; Parabacteroides;uncultured.bacterium	0.00896 [0.00401;0.01362]	0.01522 [0.01102;0.02123]	0.00721 [0.00305;0.01178]	0.74	0.12	0.02
	Firmicutes;Clostridia;Clostridiales;Peptococcaceae; Peptococcus;uncultured.organism	0.00006 [0.0;0.00013]	0.0 [0.0;0.0]	0.00000 [0.00;0.00002]	0.17	0.05	0.18
Faeces day 42 pw[4]	*Firmicutes;Clostridia;Clostridiales;Ruminococcaceae; Intestinimonas;uncultured.bacterium*	0.00001 [0.0;0.00003]	0.00005 [0.00004;0.00014]	0.00002 [0.0;0.00009]	0.39	0.01	0.23
	Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae; Alistipes;.bacterium	0.0 [0.0;0.00003]	0.00006 [0.00002;0.00014]	0.00001 [0.0;0.00004]	0.38	0.03	0.16
Faeces day 138 pw	*Firmicutes;Clostridia;Clostridiales;Lachnospiraceae; uncultured;uncultured.Firmicutes.bacterium*	2e-05 [0e+00;3e-05]	0.0 [0.0;0.0]	0.0 [0e+00;1e-05]	0.12	0.01	0.12
	Firmicutes;Clostridia;Clostridiales;Clostridiaceae.1; Clostridium.sensu.stricto.1;uncultured.Clostridiaceae.bacterium	0.00017 [0.00011;0.00026]	0.00003 [0.00002;0.00008]	0.00007 [0.00004;0.00015]	0.20	0.02	0.21
	Candidate.division.TM7;Unknown.Class;Unknown.Order; Unknown.Family;Candidatus.Saccharimonas;uncultured.bacterium	0.00045 [0.00026;0.00077]	0.00044 [0.00027;0.00060]	0.00015 [0.0;0.00028]	**0.03**	0.82	0.09
	Firmicutes;Clostridia;Clostridiales;Lachnospiraceae; Cellulosilyticum;uncultured.bacterium	0.00014 [0.00008;0.00026]	0.00064 [0.00038;0.00092]	0.00060 [0.00026;0.00105]	**0.05**	0.05	0.92
	Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides; uncultured.bacterium	0.00041 [0.00018;0.00108]	0.00142 [0.00104;0.00363]	0.00175 [0.00062;0.00431]	**0.04**	0.07	0.79
	Firmicutes;Clostridia;Clostridiales;Clostridiaceae.1; Clostridium.sensu.stricto.1;uncultured.bacterium	0.08039 [0.05077;0.11418]	0.07249 [0.06478;0.08706]	0.03572 [0.03190;0.06637]	**0.04**	0.82	0.10
	Firmicutes;Clostridia;Clostridiales;vadinBB60;uncultured.organism;	0.00229 [0.00132;0.00317]	0.00209 [0.00115;0.00271]	0.00513 [0.00302;0.00628]	**0.05**	0.82	0.08
	Firmicutes;Clostridia;Clostridiales;Christensenellaceae;uncultured; uncultured.bacterium	0.02067 [0.01509;0.03598]	0.03442 [0.01922;0.06740]	0.05353 [0.03997;0.06825]	**0.04**	0.30	0.32

Table S2.5. Relative abundance of OTUs statistically different[1] among RFI ranks (continued)

Sample type	OTU Taxonomy	Median relative abundance [confidence interval][2]			P-value[3]		
		High RFI (n=10)	Medium RFI (n=10)	Low RFI (n=12)	High vs. Low	High vs. Medium	Low vs. Medium
Caecal digesta	*Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;uncultured; uncultured.Clostridiales.bacterium*	0.00013 [0.00009;0.00034]	0.00011 [0.00004;0.00022]	0.0 [0.0;0.00003]	**0.002**	0.33	0.02
	Firmicutes;Erysipelotrichia;Erysipelotrichales;Erysipelotrichaceae; Solobacterium;uncultured.bacterium	0.00002 [0.0;0.00013]	0.0 [0.0;0.0]	0.0 [0.0;0.0]	**0.01**	0.07	0.27
	Proteobacteria;Gammaproteobacteria;Pasteurellales; Pasteurellaceae;Actinobacillus;Actinobacillus.porcinus	0.0 [0.0;1e-05]	4e-05 [0.0;6e-05]	3e-05 [1e-05;8e-05]	**0.04**	6.80E-02	0.89
Ileal Digesta	*Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;uncultured; uncultured.Clostridiales.bacterium*	0.0 [0.0;0.0]	0.0 [0.0;0.0]	2e-05 [0.0;1e-04]	0.08	0.22	0.02
	Euryarchaeota;Methanobacteria;Methanobacteriales; Methanobacteriaceae;Methanobrevibacter;uncultured.methanogenic. archaeon	1e-05 [0.0;2e-05]	5e-05 [3e-05;9e-05]	0.0 [0.0;1e-05]	0.15	0.15	0.02
	Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Oscillibacter; uncultured.bacterium	0.00002 [0.00001;0.00004]	0.00009 [0.00006;0.00011]	0.00010 [0.00005;0.00022]	**0.03**	0.01	0.93
	Actinobacteria;Actinobacteria;Corynebacteriales;Nocardiaceae; Rhodococcus;unidentified	0.37417 [0.24461;0.54878]	0.42184 [0.09561;0.54106]	0.11800 [0.03774;0.29465]	**0.04**	0.91	0.04
	Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Incertae.Sedis; Eubacterium.fissicatena	2e-05 [1e-05;2e-05]	0.0 [0.0;0.0]	1e-05 [0e+00;2e-05]	0.32	0.05	0.07
	Spirochaetae;Spirochaetes;Spirochaetales;Spirochaetaceae; Treponema;Treponema.berlinense	0.0 [0.0;0.0]	0.0 [0.0;4e-05]	3e-05 [1e-05;4e-05]	**0.02**	0.30	0.30
	Euryarchaeota;Methanobacteria;Methanobacteriales; Methanobacteriaceae;Methanosphaera;uncultured.archaeon	1e-05 [0.0;4e-05]	0.0 [0.0;0.0]	0.0 [0.0;0.0]	**0.05**	0.18	0.18
	Firmicutes;Clostridia;Clostridiales;Lachnospiraceae; Anaerosporobacter;uncultured.bacterium	1e-05 [0.0;4e-05]	0.0 [0.0;0.0]	0.0 [0.0;2e-05]	0.77	0.03	0.03
	Firmicutes;Bacilli;Bacillales;Planococcaceae;uncultured; uncultured.bacterium	0.00005 [<0.00001;0.00036]	0.0 [0.0;0.0]	0.0 [0.0;<0.00001]	0.11	0.07	0.42

[1] Statistical significance at P≤0.05; [2]The values presented are not percentages; differentially abundant OTUs for which median relative abundance was less than 0.00001 are not presented; [3]P-values in bold correspond to statistical differences between high and low RFI pigs; [4]pw: post-weaning.

Table S2.6. Effect of ranking pigs on residual feed intake (between weaning and day 126 post-weaning) on pH and volatile fatty acid (VFA) concentrations (µmol/g) in faeces at weaning, day 42 and day 138 post-weaning

	Weaning					Day 42 post-weaning					Day 138 post-weaning				
Measure	**High RFI**	**Medium RFI**	**Low RFI**	**S.E.M[1]**	**P**	**High RFI**	**Medium RFI**	**Low RFI**	**S.E.M**	**P**	**High RFI**	**Medium RFI**	**Low RFI**	**S.E.M**	**P**
pH	6.7	6.9	6.8	0.081	0.67	6.3	6.4	6.4	0.09	0.81	6.6	6.4	6.7	0.08	0.56
Total[2]	94	79	104	12.7	0.25	114	133	102	13.2	0.19	98	101	100	11.3	0.97
Acetic	26.3	23.2	30.9	3.59	0.24	36.9	44.4	33.9	4.20	0.17	35.0	36.6	39.2	4.02	0.73
Propionic	$27.2^{A,B}$	22.0^{B}	33.3^{A}	4.19	0.09	34.2	42.2	30.9	4.51	0.14	30.0	31.3	29.9	3.82	0.94
Butyric	13.0	10.3	12.2	2.27	0.67	23.2	24.5	19.7	3.42	0.53	15.2	15.8	15.1	2.35	0.97
Valeric	7.0	6.0	8.2	1.12	0.25	7.2	7.6	6.0	0.92	0.28	5.0	5.3	4.9	0.67	0.87
Isovaleric	13.3	9.8	10.8	1.83	0.39	7.6	8.8	7.1	1.03	0.42	7.7	7.5	7.1	0.98	0.88
Isobutyric	7.1	6.3	7.8	1.15	0.57	4.4	5.1	4.1	0.62	0.42	4.5	4.4	4.2	0.59	0.93

High RFI: n=10, medium RFI: n=10, low RFI: n=12.

[1]Least square means and pooled standard error of the mean are presented.

[2]Total: Sum of measured acids.

[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P \leq 0.05$).

Table S2.7. Effect of ranking pigs on residual feed intake (between weaning and day 126 post-weaning) on pH and volatile fatty acid (VFA) concentrations (µmol/g) in ileal and caecal digesta at slaughter (day 139 post-weaning)

Measure	Ileal digesta					Caecal digesta				
	High RFI	Medium RFI	Low RFI	S.E.M[1]	P	High RFI	Medium RFI	Low RFI	S.E.M	P
pH	6.8	6.7	6.7	0.09	0.55	6.6	6.7	6.7	0.09	0.37
Total[2]	47	45	53	8.1	0.73	111	96	86	14.5	0.50
Acetic	25.9	21.0	31.1	4.37	0.22	40.0	33.6	33.6	5.39	0.65
Propionic	7.4	9.2	10.8	2.24	0.51	38.8	32.6	31.1	7.16	0.73
Butyric	3.8	5.4	8.3	1.51	0.14	18.5	17.2	12.6	3.36	0.46
Valeric	2.3	2.5	2.6	0.46	0.84	5.2	5.0	3.9	0.78	0.40
Isovaleric	$4.8^{a,b}$	3.4^{b}	7.2^{a}	0.96	0.02	5.5	4.7	3.9	0.81	0.34
Isobutyric	1.5^{b}	$2.6^{a,b}$	3.5^{a}	0.55	0.04	3.2	2.8	2.4	0.57	0.57

High RFI: caecal n=8, ileal n=6; medium RFI: caecal n=10, ileal n=9; low RFI: caecal n=12, ileal n=9.
[1]Least square means and pooled standard error of the mean are presented.
[2]Total: sum of measured acids.
[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P \leq 0.05$).

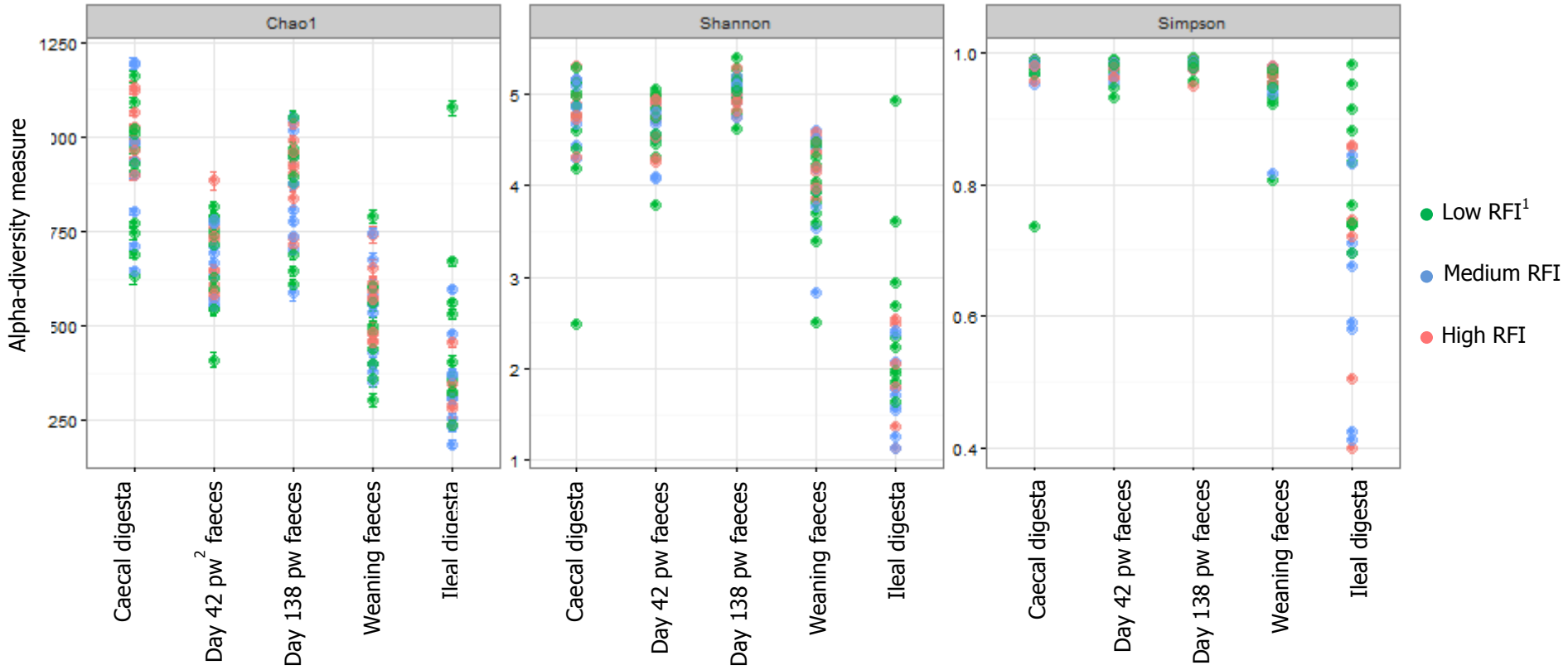
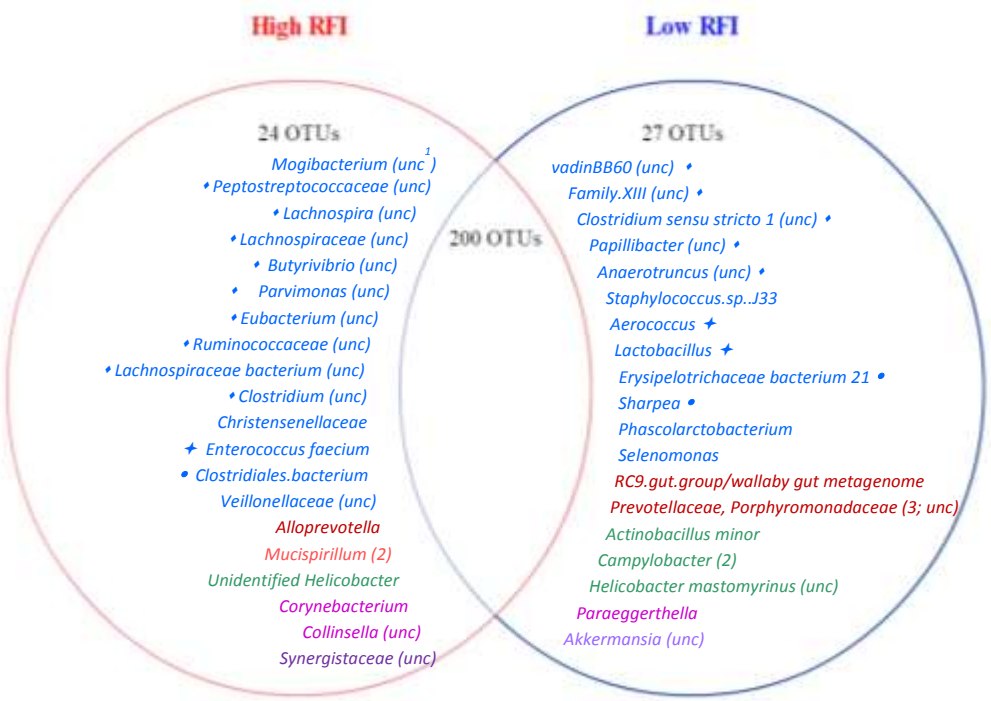


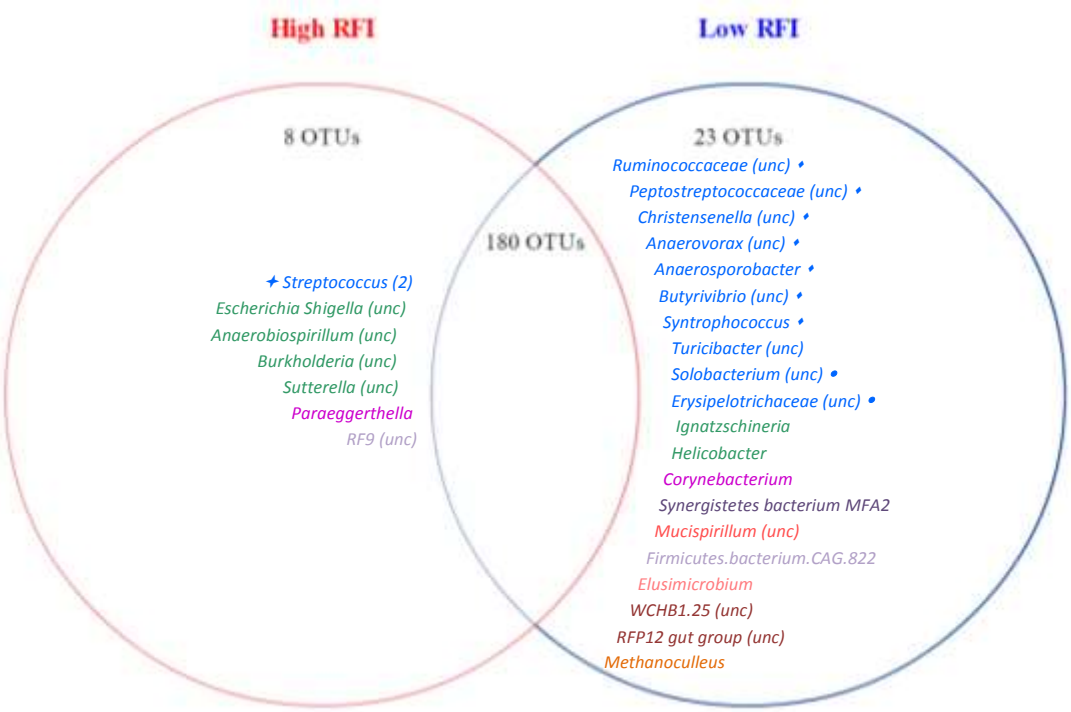
Figure S2.1. α-diversity indices of the pig intestinal microbiota according to residual feed intake (RFI) rank and sample type (n=150) (colour coded by RFI rank)

[1]RFI: residual feed intake; [2]pw: post-weaning. Low RFI: faeces (n=36), digesta (caecal n=12, ileal n=9); medium RFI: faeces (n=30), digesta (caecal n=10, ileal n=9); high RFI: faeces (n=30), digesta (caecal n=8, ileal n=6).

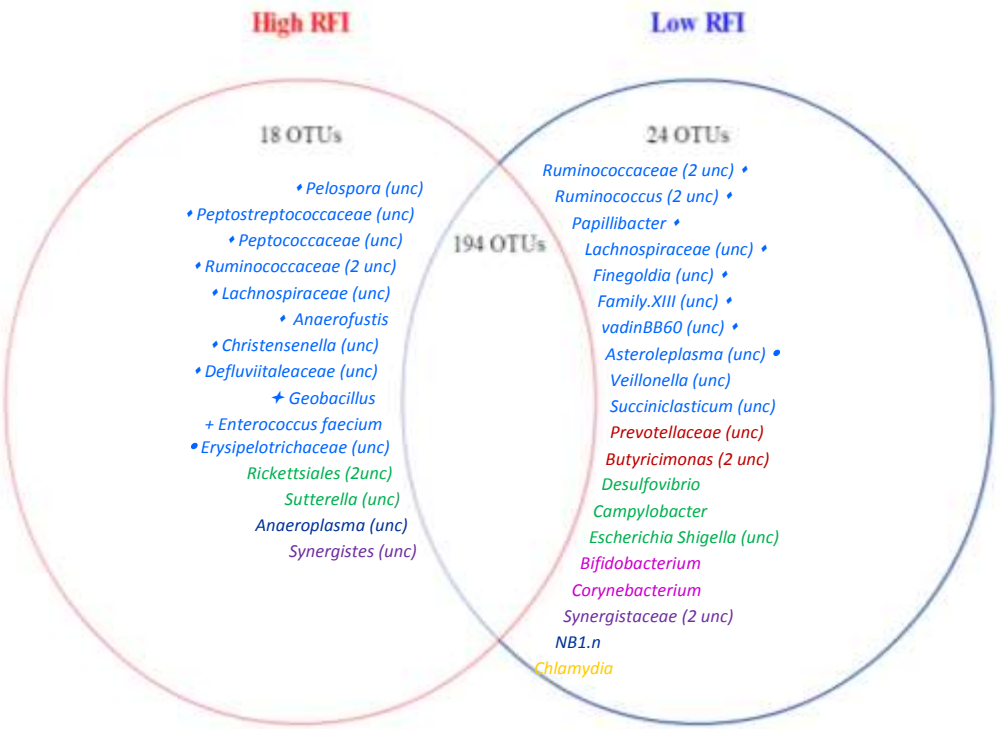
A. Weaning faeces



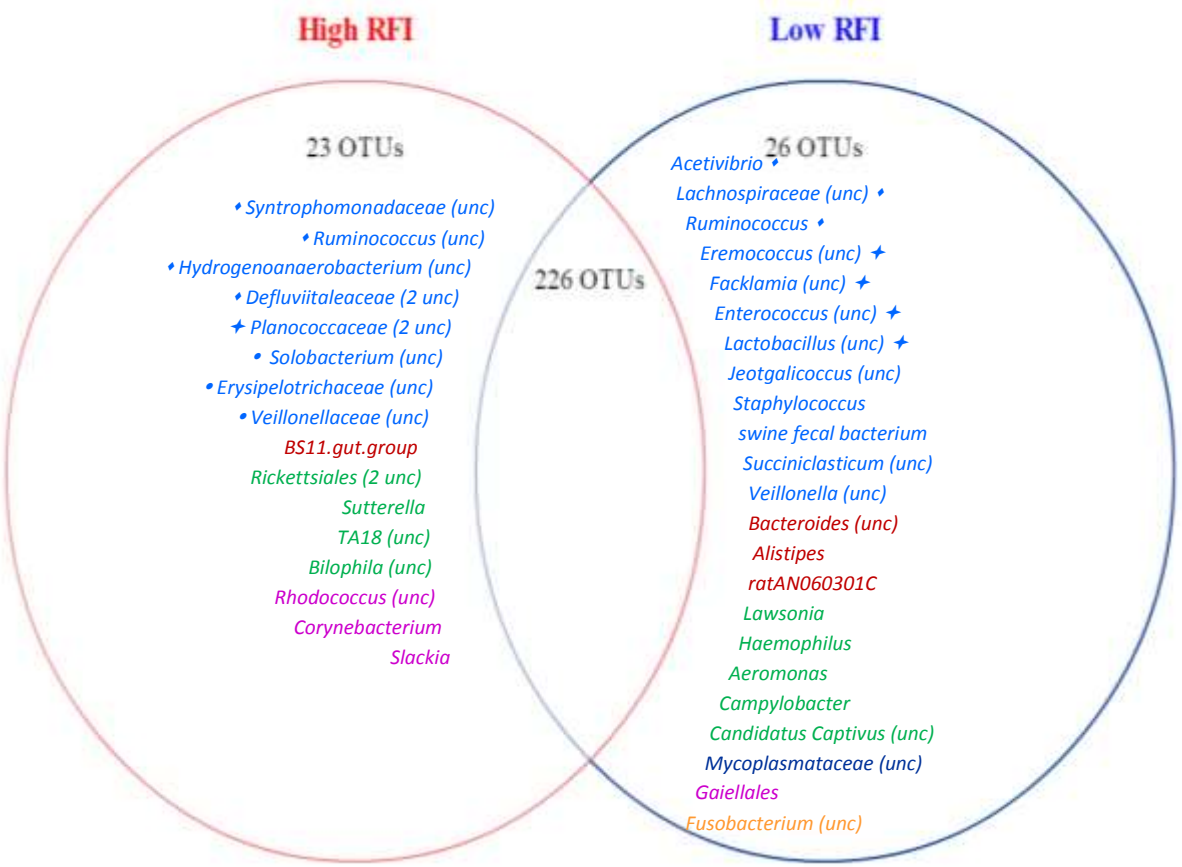
B. Day 42 pw[2] faeces



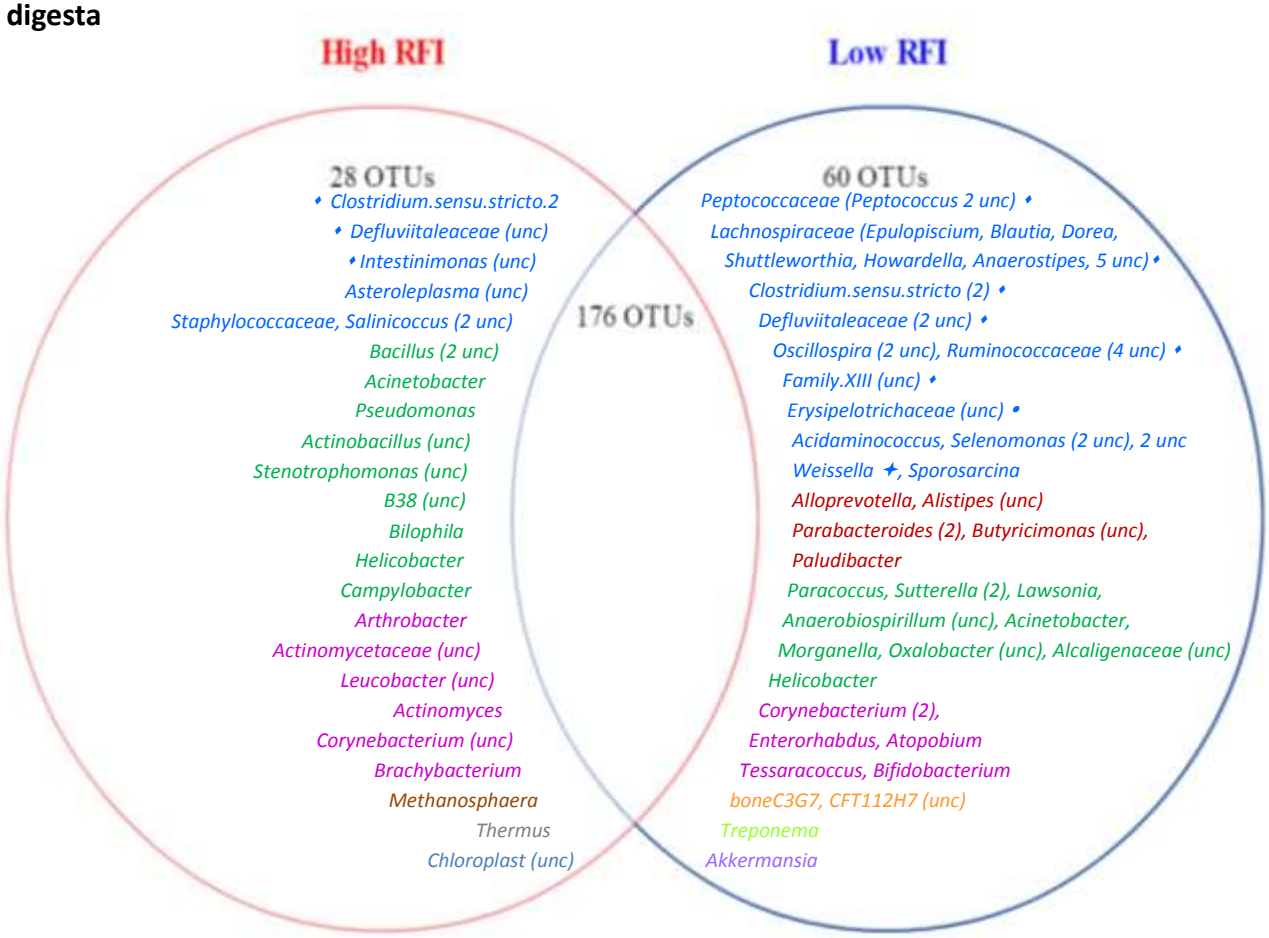
C. Day 138 pw faeces



E. Caecal digesta



D. Ileal digesta



Firmicutes ***Bacteroidetes*** ***Proteobacteria*** ***Actinobacteria*** ***Verrucomicrobia*** ***Deferribacteres***
Synergistetes ***Tenericutes*** ***Elusimicrobia*** *Lentisphaerae* *Euryarchaeota* *Chlamydiae* *Fusobacteria*
Candidate division TM7 *Spirochaetae* *Cyanobacteria* *Deinococcus Thermus*

♦ ***Clostridia/Clostridiales*** ✦ ***Bacilli/Lactobacillales*** •
Erysipelotrichia/Erysipelotrichales

Figure S2.2. OTUs found exclusively in either high or low residual feed intake (RFI)-ranked pigs across all faecal time points (A-C) and for both intestinal locations (D, E), as well as those shared across RFI ranks (80% of the OTUs were shared at weaning, 85% at day 42 pw, 82% at day 138 pw and in the caecal digesta and 66% in the ileal digesta)

Low RFI: faeces (n=36), digesta (caecal n=12, ileal n=9); medium RFI: faeces (n=30), digesta (caecal n=10, ileal n=9); high RFI: faeces (n=30), digesta (caecal n=8, ileal n=6).

[1]unc: uncultured; [2]pw: post-weaning. Numbers in parentheses denote the number of OTUs present for a given taxon where there was more than one.

3. Porcine feed efficiency (FE)-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake (RFI)

UM. McCormack, T. Curião, BU. Metzler-Zebeli, E. Magowan, D. Berry, H. Reyer, ML. Prieto, M. Harrison, N. Rebeiz, SG. Buzoianu, F. Crispie, PD. Cotter, O. O'Sullivan, GE. Gardiner, PG. Lawlor. 2017. Porcine feed efficiency (FE)-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake (RFI).

(Submitted to *Microbiome* December 2016)

3.1. Abstract

Achieving optimal feed efficiency (FE) is important in pig production for both economic and environmental reasons. The intestinal microbiota contributes to nutrient utilization, and previous research from our group has identified FE-associated taxa within the intestinal microbiota of growing pigs. The present study aimed to investigate whether FE-associated enterotypes and selected FE-associated physiological traits in pigs were consistent across the Republic of Ireland [two batches of pigs; ROI1 (medium health) and ROI2 (high health)], Northern Ireland (NI), and Austria (AT), where differences in genetic, dietary and management factors were minimized. Pigs (n=369) were ranked on divergence in residual feed intake (RFI, a metric for FE) and 100 extremes were selected in total (50 high RFI and 50 low RFI) across geographical locations for analysis of the intestinal microbiota and a range of FE-associated traits. Intestinal microbial diversity, determined by 16S rRNA amplicon sequencing, varied due to geographical location, health status and intestinal site, but not by RFI. For the high health batch of pigs (ROI2), low RFI (more feed efficient) animals had greater species diversity in the ileal and caecal digesta. In addition, *Lentisphaerae*, *Mucispirillum*, *Methanobrevibacter*, *Ruminococcaceae*, *RF16* and two uncultured bacterial taxa were more abundant within the faecal and caecal microbiota of low RFI pigs in two geographical locations and / or the two ROI batches. These taxa are major contributors to carbohydrate metabolism, which was reflected in the functional predictions. Low RFI pigs had lower faecal concentrations of total, butyric, propionic, valeric and isovaleric volatile fatty acids. None of the other physiological traits measured varied according to RFI, except salivary cortisol, which tended to be lower in low RFI pigs.

The geographical location, intestinal site, and health status greatly impacted the pig gut microbiome, which in turn presents challenges when identifying consistent reliable microbial biomarkers for FE in pigs. However, seven FE-associated enterotypes were common across geographical locations and batches, and related to a potentially "healthier" and metabolically more capable microbiota. These taxa could therefore be employed as biomarkers for FE and may merit consideration for use as probiotics or targeting by dietary means as a strategy for improving FE in pigs in the future.

3.2. Introduction

Feed efficiency (FE) is critical in the pig industry, as feed accounts for up to 75% of production costs (Teagasc, 2016). As a result, in recent years considerable attention has been focused on finding biomarkers for FE in pigs. Those that have been suggested include cortisol, a hormone which is secreted in response to stress and low blood-glucose levels, affecting metabolism of carbohydrates, lipids and protein (Hillmann et al., 2008). Animals with a higher level of serum cortisol are more likely to divert energy away from lean meat deposition, resulting in poorer FE (Richardson et al., 2004). Haematological parameters and blood markers (e.g. protein, triglycerides, cholesterol) are functional indicators of metabolic pathways, that ultimately denote homeostasis or dysfunction (Pond, 2001), and some of these have also been linked with FE in pigs (Mpetile et al., 2015, Grubbs et al., 2016). Immune status is another important factor associated with FE; it has been suggested, for example, that pigs with enhanced FE might have a more effective immune response without diverting energy away from growth and lean meat deposition (Vigors et al., 2016a, Mpetile, 2014). More feed efficient pigs were previously shown to produce lower, but sufficiently high levels of white blood cells (e.g. lymphocytes and monocytes) (Mpetile et al., 2015), and an increased expression of antigen processing related genes (Liu et al., 2016).

The resident intestinal microbiota (specifically its diversity, composition and function), is another factor that is likely to influence FE in pigs, considering its role in host metabolism and immunity, as in Chapter 2 and other published studies (Ramayo-Caldas et al., 2016, Vigors et al., 2016a). Recently, there have been an increasing number of studies aimed at characterizing the swine intestinal microbiome, identifying major populations of bacteria associated with intestinal site, age of the pig and diet (Mach et al., 2015, Zhao et al., 2015, Kim and Isaacson, 2015a, Buzoianu et al., 2012a, Yang et al., 2016). Studies have also uncovered a major role for the intestinal

microbiota and microbial metabolites in the regulation of the immune system (Levy et al., 2016). However, very few studies to date have explored the possible link between FE in pigs and the intestinal microbiota. The work from Chapter 2 demonstrated an association between porcine intestinal microbiota composition and residual feed intake (RFI; used as a metric for FE) but was limited to one batch of pigs at a single geographical location.

Previous work in humans observed that people living in different environments have dramatically different microbial profiles (Yatsunenko et al., 2012), whereas work in cattle found that management practices appear to be more influential than geographical location (Shanks et al., 2011). Therefore, following on from Chapter 2, where a first set of FE-associated enterotypes were provided, the current study sought to determine if FE-associated microbiota are consistent in pigs raised in different environments. The objective was to investigate the intestinal microbiota composition of pigs ranked on RFI, reared at three geographical locations, using common animal breeding, diet specifications and management protocols. Other physiological parameters were also assessed and correlated with intestinal microbial composition and metabolites, in an attempt to elucidate their role in influencing FE in pigs.

3.3. Materials and methods

3.3.1. Ethical approval

The pig trials in the Republic of Ireland (ROI) were approved by the animal ethics committees of Teagasc (TAEC9/2013) and Waterford Institute of Technology (13/CLS/02) and an experimental license (number AE1932/P004) was obtained from the Irish Health Products Regulatory Authority (HPRA). The pig trial in Northern Ireland (NI) was conducted under project licences PPL 2751 and PPL 2781 obtained from the Department of Health, Social Services and Public Safety (DHSSPS) which adhere to the Animals (Scientific Procedures) Act 1986. The pig trial in Austria (AT) was approved by the institutional ethics committee and the national authority according to paragraph 26 of Law for Animal Experiments, Tierversuchsgesetz 2012 – TVG 2012 (GZ 68.205/0058-WF/II/3b/2014). All animal procedures were performed according to European Union regulations outlining minimum standards for the protection of pigs (91/630/EEC) and concerning the protection of animals kept for farming purposes (98/58/EC).

3.3.2. Animal management, performance records and sampling

Four trials were conducted across three geographical locations; ROI, NI and AT. A total of 39 sows, across all three locations (25 in ROI, 8 in NI and 6 in AT), were blocked by body weight and randomly inseminated with semen from individual boars (Hermitage Genetics, Kilkenny, Ireland). One common boar was used across the three locations, with an additional 10 boars specific to ROI, three specific to NI and another three specific to AT.

Subsequent offspring comprised 369 piglets: 218 in ROI [(two batches, one medium herd health [ROI1; n=80] and one high herd health [ROI2; n=138]), 87 in NI and 64 in AT]. All pigs were weaned at ~28 days of age and were housed in groups of

intact litters and fed *via* Feed Intake Recording Equipment (FIRE) feeders (Schauer Agrotronic, Austria). Pigs were fed the same sequence of diets (starter, link, weaner and finisher), with the same diet specifications across all three geographical locations. Water and feed were provided on an *ad libitum* basis. The ingredient and chemical composition of all experimental diets are shown in Table S3.1.

Individual body weight, back-fat (BF) depth and muscle depth (MD) were recorded every week and feed disappearance was recorded daily between day 42 and day 91 post-weaning (pw). This data was used to calculate performance indicators [average daily feed intake (ADFI), average daily gain (ADG) and feed conversion efficiency (FCE)]. Ultrasound measurements (i.e. BF and MD) were taken at the 3rd and 4th last rib using Piglog 105 (Carometec, Herlev, Denmark) for ROI; Sonoscope A5 (Keebomed, Mount Prospect, IL) for NI; and Renoco lean meater (Renoco Corporation, Minneapolis, MN) for AT. All pigs were checked at least twice daily and any showing signs of illness were treated as appropriate and the details recorded. Any pigs that were treated with antibiotics were removed from the study.

After day 91 pw, RFI for each pig was calculated and extremes for RFI were selected within litter and gender. The RFI is a measure of FE that assesses the difference between the actual feed intake and the expected feed intake, which is based on live-weight of the animal, rate of gain and body fat content (Patience et al., 2015), with low RFI animals deemed to be the most feed efficient. The RFI was calculated as the residuals from a least squares regression model of ADFI on ADG, metabolic live-weight, gender and all relevant two-way interactions, as well as the effects of back-fat and muscle-depth. Pigs were ranked as either high or low RFI, with a minimum spread of 2 standard deviations from the mean (within location and batch) between the two RFI ranks. A total of 100 pigs [60 from ROI (ROI1: 20, ROI2: 40), 24 from NI and 16 from

AT] were selected for sampling. A schematic depicting the selection of pigs is shown in Figure 3.1.

Individual faecal samples were collected following rectal stimulation at day 42 and 105 pw for all pigs selected across all geographical locations, immediately snap-frozen in liquid nitrogen and stored at -80 °C for subsequent microbiota and volatile fatty acid (VFA) analysis. Two weeks after selection for RFI extremes (~day 105 pw), pigs were slaughtered by CO_2 stunning followed by exsanguination. Hot carcass weight was recorded immediately following slaughter and was multiplied by 0.98 to obtain cold carcass weight. Kill-out percentage was calculated as [(carcass weight/body weight at slaughter) x 100]. Back-fat and muscle depth at slaughter were measured at 6 cm from the edge of the split back at the third and fourth last ribs using a Hennessy Grading probe (Hennessy and Chong, Auckland, New Zealand). Lean meat yield was estimated according to the following formula: Lean meat yield = $60.30 - 0.847\ X_1 + 0.147\ X_2$ [where X_1= back-fat depth (mm) and X_2= muscle depth (mm)]. Digesta samples were collected from the terminal ileum (15 cm before the ileo-caecal junction) and the caecum (terminal tip) from the selected pigs in ROI and AT. Samples were immediately snap-frozen in liquid nitrogen and stored at -80 °C for subsequent microbiota and VFA analysis.

3.3.3. Salivary cortisol analysis

On day 100 pw, saliva samples were collected from the pigs in ROI1 by allowing them to chew on a cotton bud (Salivette, Sarstedt, Wexford, Ireland). Cortisol concentrations were determined in the saliva samples in duplicate using a high sensitivity enzyme linked immunosorbent assay (ELISA) kit (Salimetrics, Europe Ltd, Suffolk, UK) according to the manufacturer's instructions.

3.3.4. Haematology and blood biochemistry analyses

Blood was collected during exsanguination at the slaughter plants for haematology and biochemistry analyses from pigs in ROI and AT. For haematological analysis, blood was collected in vacuette tubes (ROI: Labstock, Dublin, Ireland; AT: Sarstedt, Nürnbrecht, Germany) containing EDTA to prevent clotting, and analyzed within 4 h using a Beckman Coulter Ac T Diff analyzer (Beckman Coulter Ltd., High Wycombe, UK) for ROI pigs and a ProCyte dx Hematology Analyzer (IDEXX Laboratories, Inc., Westbrook, Maine, USA) for AT pigs.

For biochemical analysis, blood was collected from ROI pigs in vacuette tubes (Labstock, Dublin, Ireland) and allowed to clot at room temperature prior to centrifugation at 1,500 × *g* for 10 min. The serum was then collected and stored at -80 °C for subsequent analysis. Serum samples were analysed using an ABS Pentra 400 clinical chemistry analyser (Horiba, ABX, North Hampton, UK) for total protein, blood urea nitrogen, cholesterol, glucose, triglycerides, creatinine, and creatine kinase. The analyzer was calibrated according to the manufacturer's instructions and every fifth sample was run in duplicate to determine analyser accuracy. For pigs in AT, blood was collected in serum collection tubes (Sarstedt, Nürnbrecht, Germany) and placed on ice prior to centrifugation at 1,811 × *g* for 10 min. Serum was then collected and stored at -80 °C for subsequent analysis of blood urea nitrogen, glucose, triglycerides and cholesterol which were determined by standard enzymatic colorimetric analysis using a clinical chemistry auto analyser, as outlined previously (Metzler-Zebeli et al., 2015a).

3.3.5. Immunological analyses

Ileal tissue (2-3 cm) was collected 15 cm before the ileo-caecal junction from ROI1 pigs at slaughter, placed in Hank's balanced salt solution (HBSS; Sigma Aldrich, Wicklow, Ireland) and put on ice. Lamina propria lymphocytes (LPL) and intra-

epithelial lymphocytes (IEL) were isolated from the ileal tissue as previously described (Walsh et al., 2011) and pooled. Briefly, cells were isolated from the mucosa and submucosa of the porcine ileal tissue samples, suspended at 10^6 cells/mL in complete medium [IMDM plus Glutamax (Invitrogen), 20 % heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 mg/mL streptomycin] and transferred into 24 well plates (Sarstedt, Numbrecht, Germany) in quadruplicate. Cells were then pooled and treated with phosphate buffered saline (PBS, control) or stimulated with phorbol myristate acetate [(PMA, 25 ng/mL; Sigma-Aldrich) plus ionomycin (I; 1 μg/mL; Sigma-Aldrich), mitogen-stimulated] at 37 °C in a 5 % (v/v) CO_2 humidified atmosphere for 4-5 h and incubated for 18 h. Cells were centrifuged at 1,230 x *g* for 20 min and supernatants were stored at -80 °C until cytokine analysis as outlined below.

Immunophenotyping was then performed on the pooled LPL and IEL that were washed and re-suspended in 2 % FBS/PBS using a BD FACSCanto II™ flow cytometer (BD Biosciences, Devon, UK), with at least 50,000 events acquired and analyzed. Data were analyzed using FACSDIVA™ software (BD Biosciences). Primary and secondary antibodies were added at concentrations determined by previous titration and incubations were performed in the dark at room temperature for 15 min. Antibodies used were CD45 fluorescein isothiocyanate [FITC (lymphocyte marker); AbD Serotec/BioRad; Kidlington, UK] to verify white blood cell population identified by light scatter, anti-porcine B cell marker PE (Abcam, Cambridge, UK), CD3 phycoerythrin/cyanine 5 (PE/Cy5 T cell marker; Abcam), anti-porcine CD14 PE/Cy7 (monocyte marker; Abcam), mouse anti-porcine CD4a FITC (BD Biosciences), mouse anti-porcine CD8a (BD Biosciences), purified rat anti-pig γδ T lymphocytes (BD Biosciences) and goat anti-rat FITC (AbD Serotec/BioRad). Proportions of B cells, total T cells and monocytes were calculated as percentages of the total white blood cells that were identified by light scatter and verified with CD45 antibody to be 63.51±24.49

% positive in high RFI pigs, and 51.53±16.45 % positive in low RFI pigs. The T cell subsets were calculated based on the percentage of CD3 positive cells.

Concentrations of IL-4, IL-6, IL-8 and TNFα were subsequently determined in the supernatants from pelleted immune cells treated with PBS and PMA+ I by multiplex ELISA (R&D systems, Minneapolis, MN) in triplicate following the manufacturer's instructions.

3.3.6. Haptoglobin in serum

The concentration of haptoglobin was determined in serum samples collected from ROI1, AT and NI pigs using a porcine-specific commercial ELISA kit (Genway, San Diego, CA, USA) according to the manufacturer's instructions. Serum samples were diluted 7,000 to 12,500-fold, depending on the actual haptoglobin concentrations in samples. Haptoglobin concentrations were determined in duplicate and the intra-assay coefficient of variation was below 10%.

3.3.7. Lipopolysaccharides in caecal digesta

Concentrations of cell-free lipopolysaccharides (LPS) in caecal digesta collected from all pigs were determined using the pyrochrome *Limulus* amebocyte lysate (LAL) assay (Associates of Cape Cod Inc., East Falmouth, MA) as previously described (Metzler-Zebeli et al., 2013). After dilution and de-proteinisation by heating, the supernatants were used in the assay. Changes in optical density of samples at 405 nm were measured against calibration curves using Pyros EQS software (Associates of Cape Cod Inc.) after addition of pyrochrome LAL reagent and incubation at 37 °C. Reactions were run in duplicate and the intra-assay coefficient of variation was <10 %.

3.3.8. Microbiota profiling

Total DNA was extracted from faecal, ileal and caecal samples using the QIAamp DNA stool minikit (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions, apart from adding a beat beating step, and increasing the lysis temperature to 95 °C, to increase DNA yield (Buzoianu et al., 2012a).

Microbial profiling was performed using high-throughput sequencing of the V3-V4 region of the 16S rRNA gene (paired-end reads of 2x250 bp) on an Illumina MiSeq platform. The Illumina-recommended 16S-metagenomic library preparation (Nextera) protocol was followed, except that the PCR mix volume was doubled in the first PCR step, and amplification cycles were increased to 30 instead of 25. Any samples with less than 40,000 post quality reads on the MiSeq were removed from the analysis. Raw sequences were merged using Flash (with a minimum overlap of 30 bp and a minimum read length of 460 bp) and quality checked using the split libraries script (with default parameters) from the QIIME package version 1.9.1. Reads with a 97 % sequence homology were clustered into operational taxonomical units (OTUs) by de novo OTU picking and chimeras removed with the 64-bit version of USEARCH (Edgar, 2010). Subsequently, OTUs were aligned to the SILVA rRNA specific database (version 111) and a phylogenetic tree was generated within QIIME. Alpha and beta diversity analyses were also performed using QIIME. Principal coordinate analysis (PCoA) plots based on unweighted Unifrac distances were visualised using EMPeror v0.9.3-dev. Further downstream images were generated using the R package Phyloseq.

3.3.9. Microbial function prediction

The predicted functionality of the microbiota for each sample based on 16S rRNA data was determined using PICRUSt according to RFI rank, geographical location and sample type. The PICRUSt is a tool which uses the 16S rRNA gene sequence, to predict the functionality of microorganisms (Langille et al., 2013).

Prediction of functions was inferred based on Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2008) and Clusters of Orthologous Groups of proteins (COGs) annotations, which assigns annotations according to the database. KEGG orthology (KO) functions not bacteria-related or for which the relative abundance was <0.01 % for all of the RFI ranks were dismissed.

3.3.10. Volatile fatty acid concentrations in faeces and digesta

Volatile fatty acid concentrations (i.e., acetic, butyric, isobutyric, propionic, valeric and isovaleric acids) were measured in triplicate in faeces collected on days 42 and 105 pw, in ileal and caecal digesta from the ROI1 pigs and in caecal digesta from AT pigs using gas chromatography (ROI1: Agilent 5890 gas chromatograph, AT: Fisons gas chromatograph Model 8060 MS DPFC). For samples analysed in ROI, ~8 g of sample was weighed and diluted with 5 % Trichloroacetic acid (TCA, 2.5 x weight of sample) and centrifuged at 1,800 × *g* at 4 °C for 10 min. One and a half mL of the resultant supernatant and 1.5 mL internal standard (0.043 M of 3-methylvaleric acid in 0.15 M of oxalic acid, Sigma Aldrich) were mixed gently, and the mixture filtered through a 0.45 µm filter (VWR International Ltd, Blanchardstown, Dublin 15, Ireland) into a labelled 8 mm amber GC vial (Antech Solutions Ltd., Waterford, Ireland) and stored at -80 °C until analysed as previously described (Prieto et al., 2014).

For caecal digesta analysed in AT, aliquots of 1 g were thawed on ice and mixed with 0.2 mL metaphosphoric acid (4.3 M; Sigma-Aldrich), 1 mL of double-distilled water, and 200 µL internal standard (0.024 M of 4-methylvaleric acid in 4.3 M of phosphoric acid, Sigma Aldrich), and the mixture was centrifuged at 3,148 × *g* for 10 min. The clear supernatant was filtered through a 0.45 µm filter (VWR International Ltd) into a labelled 8 mm GC vial and analyzed as previously described (Metzler-Zebeli et al., 2015b).

3.3.11. Statistical analyses

Growth performance parameters (weight, ADG, ADFI, FCE) were analysed using a mixed linear model in SAS 9.3 (SAS, 2011). Fixed effects included in the model were RFI rank, geographical location, gender and time period, as well as their interactions. While adjusting for sex, sow was included as a random effect, and a repeated measures model was used to describe correlations between time periods. Physiological parameters measured at only one time point (i.e. ileal lymphocyte populations and cytokine data, salivary cortisol, biochemical, haematological, haptoglobin and VFA concentrations) were analyzed using a mixed linear model also; the aforementioned fixed effects were included in the model. Comparisons of means were undertaken using a Tukey correction for multiple testing. Residual diagnostics were made to ensure that the assumptions of the analysis were met.

Statistical differences for microbiota abundance at a phylum and genus level were calculated in R (R Team, 2010) using the SILVA 16S specific database (version 111) and were estimated using the Kruskal-Wallis test for independent samples and the Wilcoxon-Rank test for paired samples. Corrections for multiple comparisons were made using the Benjamini-Hochberg method.

The RFI value, and VFA concentrations found to differ between low and high RFI pigs were correlated with the taxonomic relative abundances for every sample (at all time points, for each geographical location where appropriate). Spearman correlation values were calculated in SAS, using the PROC CORR procedure, and P-values were adjusted using the Stepdown Bonferroni test.

For all statistical analysis conducted, significance was set at $P \leq 0.05$.

3.4. Results

3.4.1. Pigs ranked as low or high RFI at different geographical locations had distinct RFI values

Growth performance parameters, including RFI, were recorded from day 42 to day 91 pw and presented in Table 3.1. Across geographical locations, there was a distinct separation between high and low RFI pigs ($P<0.001$). Average daily gain ($P=0.25$) and weight at day 42 pw ($P=0.22$) did not differ between RFI rank across locations, but weight at day 91 pw ($P<0.001$), ADFI ($P<0.005$) and FCE ($P<0.03$) did.

3.4.2. Microbial diversity was affected by age of the pig and geographical location but not by RFI rank, except for species diversity in the digesta of ROI2 pigs

Microbial richness and diversity within the faeces (both at day 42 pw and 105 pw) were not associated with RFI rank (data not shown). However, in the ileal and caecal digesta from ROI2, species diversity, depicted by the Shannon and Simpson α-diversity indices, was higher for low RFI pigs ($P<0.05$; Figure 3.2).

Overall, ß-diversity of the intestinal microbiota was affected by sample type (i.e. faeces and / or digesta), including the age of the pig, but not by RFI rank (Figure 3.3). At each faecal time point and digesta type, location-specific effects were observed, with samples from the same geographical location generally clustering together in the PCoA plots ($P<0.001$, Figure 3.4). However, one exception was that, samples from ROI1 and ROI2 pigs did not cluster together, even though they originated from the same location. In fact, the microbial diversity of samples from ROI1 pigs were closer to that of samples from AT pigs and the pigs from ROI2 were more similar to samples from pigs in NI.

Sequence proportions for taxonomic groups from phylum down to genus level were examined. *Firmicutes* was the most abundant phylum in the faeces, and in the

ileal digesta, in pigs from all geographical locations (Figure S3.1). The ileal samples had a greater abundance of *Firmicutes* and a much lower abundance of *Bacteroidetes*, compared to the faecal and caecal samples. Pigs from ROI1 had a greater abundance of *Tenericutes* in the ileum, compared to pigs in ROI2 and AT ($P<0.05$; Figure S3.1).

When the intestinal microbiota composition of high versus low RFI pigs were compared, a total of 192 compositional differences, mostly for taxa at low relative abundance, were found between high and low RFI pigs across all geographical locations ($P<0.05$). Most RFI-associated differences were observed in ROI2 pigs (105 taxa vs. 59 taxa in ROI1 pigs, 11 taxa in NI pigs and 17 taxa in AT pigs). Phylum-level differences between high and low RFI pigs are shown in Figure S3.1, and included *Firmicutes* (ROI1; day 105 pw and caecal digesta; ROI2; ileal digesta), and *Proteobacteria* (ROI1; caecal digesta). A number of low relative abundant phyla differed significantly between RFI ranks: *Verrucomicrobia* (ROI1; day 42 and day 105 pw, ROI2; caecal digesta), *SHA.109* (ROI2; day 42 pw), *Deferribacteres* (AT; day 42 pw), *Lentisphaerae* (ROI1 and ROI2; day 105 pw), *Euryarchaeota* (ROI1; day 105 pw), *Candidate division TM7* (ROI2; day 105 pw), *Cyanobacteria* (ROI2; ileal digesta and AT; caecal digesta), and *Planctomycetes* (ROI2; ileal digesta). For the majority of phyla, an increase in relative abundance was observed in the low RFI (i.e., more feed efficient) pigs compared to their high RFI counterparts, except for *Firmicutes* and *Verrucomicrobia* in ROI1 at day 42 pw, for which a drop in abundance was observed in low RFI pigs.

3.4.3. RFI-associated bacterial taxa within the porcine intestinal microbiota are common across geographical locations

Although none of the 192 RFI-associated taxonomic differences within the faecal and / or intestinal microbiota were common to all four trials, seven taxa were found to be enriched in low RFI (more feed efficient) pigs on more than one geographical location or from different batches reared in ROI (Figure 3.5, $P<0.05$). In the faeces

collected on day 42 pw, *Mucispirillum* (from *Deferribacteres*) were more abundant in low compared to high RFI pigs from both NI (2-fold) and AT (15-fold) ($P<0.05$). At day 105 pw, the phylum *Lentisphaerae* was enriched in the faeces of low RFI pigs in both ROI1 and ROI2 (ROI1: 2.4-fold, ROI2: 1.7-fold), as was the genus *Methanobrevibacter* (from *Euryarchaeota*; ROI1: 2.3-fold, ROI2: 3.3-fold, $P<0.05$). In the caecal digesta, we found four cross-locational RFI-associated taxa. In both ROI1 and ROI2, the bacterial family RF16 and an uncultured bacterium from the same family were enriched in low RFI pigs (ROI1; 22-fold, ROI2; 5-fold and ROI1; 14-fold, ROI2; 4-fold, respectively; $P<0.05$). The RF16 family was also 20-fold more abundant in the faeces of low RFI compared to high RFI pigs at day 105 pw in ROI1 ($P<0.05$, data not shown). In addition, two similar OTUs belonging to an uncultured bacterium from RF16 were found at higher abundance (~20 fold) in the faeces at day 105 pw in low RFI pigs from both ROI1 and AT compared to their high RFI counterparts ($P<0.05$). Furthermore, in the caecum, low RFI pigs in ROI2 and AT had a higher abundance of the family *Ruminococcaceae* (1.2-fold and 1.1-fold, respectively) and an uncultured bacterium from *Cyanobacteria* (10-fold and 1.5-fold, respectively). In contrast, in the ileum, no common RFI-associated differences were observed across geographical locations.

3.4.4. Predicted microbial pathways associated with RFI were mainly related to core metabolism with only a few common between ROI pigs

Functionality of the intestinal microbiota was inferred using the PICRUSt package. One hundred and two microbial pathways differed significantly in relative abundance between high and low RFI pigs across geographical location and by sample type and were subsequently grouped into major functional categories (Figure S3.2). These pathways were present at very low relative abundance (≤ 2.1 %) and were mainly related to metabolism, especially carbohydrate metabolism in the faeces at day 42 pw,

energy metabolism at day 105 pw and in the caecum, and nucleotide metabolism in the ileum (Figure S3.2). Depending on the age of the pig, there was also a relatively high representation of pathways related to genetic information processing (e.g. replication and repair, transcription). There were differences in the abundance of KO functions between high and low RFI pigs across locations. In the caecal and ileal digesta, for most of the locations, a number of pathways were more abundant in the low RFI pigs than in their high RFI counterparts. In the faeces at day 42 pw, most of the differentially abundant pathways were found in the pigs from NI, whereas for the rest of time points, most of the differences were assigned to ROI1 and ROI2 pigs. However, none of the differentially abundant predicted microbial pathways were common to all geographical locations and only three followed the same trend in both ROI batches; biosynthesis of fatty acids in the faeces at day 105 pw and inositol phosphate metabolism and porphyrin/chlorophyll metabolism in the caecal digesta (Figure 3.6). The latter was most abundant in the high RFI (less feed efficient) pigs, while the other two pathways were marginally higher in the low RFI pigs. The inositol phosphate metabolism pathway, belonging to the core carbohydrate metabolism function, was also differentially abundant in both the ileal and caecal digesta of pigs from both ROI batches.

3.4.5. Low RFI pigs had lower concentrations of volatile fatty acids in the faeces at slaughter age

Concentrations of VFAs were determined in faeces collected on days 42 and 105 pw, and in the ileal and caecal digesta from pigs in ROI1 as well as in the caecal digesta from pigs in AT (Figure 3.7). No differences were observed between RFI ranks in faeces collected on day 42 pw. However, low RFI (more feed efficient) pigs from ROI1 had lower concentrations of total VFAs, as well as lower butyric and propionic acids ($P<0.05$), as well as a tendency for lower valeric ($P=0.07$) and isovaleric acid ($P=0.09$)

concentrations in the faeces collected at day 105 pw. In the ileal digesta of ROI1 pigs, low RFI pigs had higher concentrations of total VFAs and acetic acid ($P<0.05$). In the caecum, there was a strong influence of geographical location observed for all VFAs measured, apart from butyric and isobutyric acid. Pigs in AT had higher concentrations of total VFAs, as well as higher acetic and propionic acid concentrations, compared to ROI1 pigs ($P<0.05$). The pigs in AT had lower concentrations of isovaleric acid compared to pigs from ROI1 also ($P<0.05$) Valeric acid was lower in low RFI pigs from ROI1 compared to their high RFI counterparts, and compared to pigs from AT ($P<0.05$).

3.4.6. *Verrucomicrobia* was correlated with low RFI value, and an uncultured genus was correlated with valeric acid

A correlation analysis was performed between the intestinal microbiota composition, at the phylum and genus levels, with RFI value and VFAs that differed significantly between RFI ranks, and those that were significant are shown in Figure S3.3. None of the phyla or genera that were RFI-associated in this study were significantly correlated with RFI value. At the phylum level, only *Verrucomicrobia* was correlated with a low RFI value (negative correlation $R=-0.56$; $P<0.05$). At the genus level, *Megasphaera* ($R=-0.57$) and an uncultured genus from *Rhodospirillaceae* ($R=-0.54$) were correlated with a low RFI value (negative correlation; $P<0.05$).

In relation to correlations performed with VFA concentrations, butyric acid in the faeces collected from ROI1 pigs at day 105 pw was positively correlated with *Mycoplasma* ($R=0.69$), but negatively correlated with *Pyramidobacter* ($R=-0.59$; $P<0.05$). Valeric acid was negatively correlated with an uncultured genus from *Verrucomicrobia* subdivision 5b ($R=-0.70$), but isovaleric acid was positively correlated with *Collinsella* ($R=0.67$; $P<0.05$). In the ileum of ROI1 pigs, there was a negative

correlation observed between acetic acid and *Clostridium sensu stricto* 1 (R=-0.70; P<0.05)

3.4.7. Salivary cortisol, but not haematological and biochemical parameters, differed according to RFI rank

Salivary cortisol, measured in pigs from ROI1, tended to be lower in low RFI (more feed efficient) pigs (P=0.06; Table S3.2). However, in pigs from ROI1, ROI2 and AT no significant differences were observed according to RFI for serum biochemistry measures, nor for any of the haematological parameters measured (P>0.05; Table S3.2 and Table S3.3), and most were within the normal ranges as previously reported in growing pigs (Klem et al., 2010).

3.4.8. No RFI-associated differences were found in ileal immunological response, serum haptoglobin or caecal LPS levels

No significant differences were observed between high and low RFI pigs from ROI1 for any of the ileal lymphocyte populations or cytokine concentrations measured from these isolated LPL and IEL, either control or mitogen-stimulated (Table S3.4). However, most of the lymphocyte populations in pigs from both RFI ranks were within the range previously recorded for younger, healthy/control pigs (Walsh et al., 2011, Nofrarias et al., 2006). Similarly, no significant differences were observed between RFI ranks, across geographical locations for serum haptoglobin concentrations (P>0.05; Table S3.5). There were also no significant differences in LPS concentrations measured in the caecal digesta of all pigs (Table S3.5).

3.5. Discussion

Very little information is available regarding the possible contribution of the gut microbiome to FE in pigs. Although Chapter 2 did demonstrate an association between porcine intestinal microbiota composition and RFI (as a metric for FE), it was limited to one batch of pigs at a single rearing site. Following on from this, the intestinal microbial profile of pigs ranked on RFI from three geographical locations was assessed in the present study in order to expand our knowledge of RFI-associated intestinal microbiota and to attempt to find reliable microbial biomarkers for FE in pigs. Across geographical location, there were fewer potential microbial biomarkers identified and most of these were different to those pinpointed in our earlier study. However, the fact that ranking on RFI was performed during different stages of the production cycle, i.e. day 42 to 91 pw in the current study compared to between weaning (28 days old) and day 126 pw in the previous study, could help to explain the apparent discrepancies between studies. Moreover, in the present study, none of the RFI-associated differences in intestinal microbiota composition found were common across all geographical locations. Similarly, studies in chickens have highlighted the challenge of finding common FE-associated microbes across locations (Siegerstetter et al., 2016) and even across batches from within the same location (Stanley et al., 2016).

The limited cross-locational RFI-associated differences in the intestinal microbiota were perhaps due to variation in the core microbiome in pigs in each location, as evidenced by the location-specific diversity revealed by the PCoA plots. Although it has previously been shown that poultry raised in different environments have different microbiota (Stanley et al., 2016), to date this has not been shown for pigs. However, this seems to be the case in the present study, even though external factors including diet, genetics and management protocols were controlled. Differences in health status may also be responsible, as for instance, the highest number of RFI-

associated compositional differences was found in ROI2 pigs, which were deemed to represent a high herd health status. This is likely reflective of a higher microbial diversity, as seen within the caecal and ileal microbiota of the low RFI (more feed efficient) pigs from that batch. Indeed, in humans, lower microbial diversity has been associated with gastrointestinal disorders and obesity (Pozuelo et al., 2015, Le Chatelier et al., 2013). Overall, environmental influences and inter-individual variability (Stanley et al., 2016) make it difficult to find reliable biomarkers and ultimately targeted feed additives e.g. probiotics/prebiotics to enhance FE in pigs.

In the present study, the overall intestinal microbiota composition showed a high abundance of *Firmicutes* and *Bacteroidetes* which mirrors what is commonly identified as major constituents of the core pig microbiome (Xiao et al., 2016). A lower relative abundance of the phylum *Candidate division TM7* and genus *Candidatus Saccharimonas* observed in the low RFI pigs from ROI2 at day 105 pw resembles what was found in Chapter 2. This phylum is diverse, comprising ubiquitous members with potential pro-inflammatory activity as it was previously found to be enriched during inflammatory bowel disease (Ferrari et al., 2014).

Although none of the RFI-associated differences in microbiota composition were common to all geographical locations, seven taxa were found to differ in their abundance between high and low RFI pigs at more than one geographical location or from different batches reared at the same location (i.e. ROI). Although, within RFI-rank, variation between pigs were observed (as indicated by the box plots shown in Figure 5), these taxa differences do appear to explain, at least in part, differences in FE and could potentially be used as microbial biomarkers for FE. For example, the low RFI (more feed efficient) -associated enterotypes have a major role in core metabolism of carbohydrates. In faeces collected at day 42 pw, *Mucispirillum* which is a mucin

degrader, was found to be higher in low RFI pigs from NI and AT. However, too much of a shift towards this genus could be harmful, causing disruption of the mucus layer (Belzer et al., 2014). In faeces collected at day 105 pw, *Methanobrevibacter* was more abundant in the highly efficient compared to the poorly efficient pigs in both ROI batches. This genus has previously been positively correlated with crude fibre digestibility in pigs (Niu et al., 2015) and also plays an important role in methane production (Pimentel et al., 2012). Recently, it was found to be enriched in healthy humans compared to those with irritable bowel syndrome (Pozuelo et al., 2015). A link with leaner phenotypes in humans is also noteworthy (Million et al., 2012). The phylum *Lentisphaerae*, enriched in low RFI pigs, has previously been linked to improved health in humans, which might suggest a healthier gut microbiome (Jiang et al., 2015). This phylum has also been previously associated with improved FE and, specifically, weight gain, albeit in cattle (Myer et al., 2015), which would suggest a benefit in further investigation of the role of *Lentisphaerae* in improving FE in pigs. In the caecal digesta, *Ruminococcaceae,* which have a central role in the fermentation of carbohydrates, including cellulose (Salonen et al., 2014, David et al., 2014), was identified as a RFI-associated taxon in pigs from both AT and ROI2. Lastly, an-uncultured bacterium from the RF16 family, along with the RF16 family itself (from *Bacteroidetes*) were both higher in relative abundance in low RFI pigs. Although there is little information regarding the role of bacteria from this family within the intestinal community, they have been previously found to be enriched in pigs fed a diet high in complex carbohydrates (Sun et al., 2015).

Recently, a metagenomic catalogue of the pig gut microbiome has demonstrated a predominance of bacterial genes with functional relation to metabolic pathways and genetic information processing (Xiao et al., 2016). In the present study, the RFI-associated predicted microbial function agreed with the compositional data, showing

that most predicted pathways enriched in low RFI pigs were related to core metabolism, including carbohydrate, energy and nucleotide metabolism. Furthermore, microbial metabolites such as VFAs have a key role in modulation of host cells, as they constitute an extra source of energy for the host. The concentration of VFAs present impacts the host phenotype, with for example, higher concentrations of VFAs in the faeces associated with obesity (Fernandes et al., 2014). The fact that faecal VFA concentrations were lower in low RFI pigs suggests that improved colonic absorption occurred in these pigs, indicating better utilisation of bacterial fermentation end-products in the large intestine(Williams, 2001). There appeared to be a strong influence of rearing environment for the caecal VFAs measured in the present study, with ROI1 pigs having lower concentrations of most of the VFAs measured compared to AT pigs.

Apart from the microbiota, salivary cortisol (lower in low RFI pigs) was the only other RFI-associated measure observed and could therefore be utilised as a biomarker for improved FE in pigs, similar to what was previously suggested in cattle (Richardson et al., 2004). No differences in serum biochemical measures were observed and these were within the normal reference values for growing pigs . Despite the absence of a link between ileal immune-competence and FE in our study, an adequate number of ileal immune cells (i.e. within normal ranges previously found for healthy, control pigs (Nofrarias et al., 2006, Walsh et al., 2011)) indicate an ability to fight off disease, while maintaining optimum growth performance. Likewise, no RFI-associated differences were found for caecal concentrations of the bacterial endotoxin, LPS, a potent immunogen, high serum concentrations of which have previously been linked to poor FE (Mani et al., 2012). Serum concentrations of the acute phase protein haptoglobin also did not differ in the present study, although previously found to be lower in low RFI pigs (Mani et al., 2013). Although previous work has shown that low RFI pigs were more efficient in terms of producing lower, but sufficient levels of blood

lymphocytes, monocytes, and white blood cells compared to high RFI pigs (Mpetile et al., 2015), no RFI-associated haematological differences were found in the present study.

3.6. Conclusions

In conclusion, the FE-associated bacterial taxa consistently found across different geographical locations and batches may have a role to play in improving FE in pigs, mainly because of their importance in relation to carbohydrate metabolism. Besides this, we showed methanogenic members of *Archaea* (*Methanobrevibacter*) as likely implicated in shaping FE in pigs. In the future, these FE-associated taxa can potentially be used as probiotics or targeted by dietary means as a strategy for improving FE in pigs. Alternatively, they could be exploited as potential predictive biomarkers for porcine FE. However, the unculturable nature of many of these taxa, together with location-specific findings highlight the challenges associated with translation of our results into a set of reliable usable biomarkers and/or potential probiotics for pigs. Furthermore, the complex interplay between enterotypes within the gut ecosystem makes the cause-effect relationship intricate, and the microbial taxa identified in the present study cannot be interpreted as definitive determinants for FE in pigs, without additional studies.

3.7. Tables and Figures

Table 3.1. Growth performance of pigs ranked on residual feed intake (RFI) at three geographical locations

Parameter	High RFI				Low RFI				SEM[1]	P-value		
	ROI1[2]	ROI2[3]	NI[4]	AT[5]	ROI1	ROI2	NI	AT		RFI*location	RFI	Location
RFI (g/day)	1237[a]	1108[a]	207[b]	1171[a,b]	-1030[c]	-737[c]	-200[c]	-956[c]	227.6	<0.001	<0.001	0.86
Weight day 42 pw[6] (kg)	32.2	33.3	27.4	30.2	33.6	31.9	28.7	28.6	1.93	0.22	0.97	0.27
Weight day 91 pw (kg)	83.1[b,c]	82.3[b,c]	91.3[a,c]	85.2[a,b,c]	87.2[a,b,c]	79.5[b]	95.2[a,]	82.8[b,c]	1.94	<0.001	0.83	0.35
ADFI[7] (g/day)	2025[b,d]	2194[a,d]	2033[a,b]	2380[a]	1988[b,d]	1851[b,c]	1586[c]	1942[b,c,d]	74.1	0.005	<0.001	0.005
ADG[8] (g/day)	1028	902	891	1131	1065	847	894	1077	30.0	0.25	0.44	<0.001
FCE[9] (g/g)	2.05[b]	2.62[a]	2.40[a]	2.11[b]	1.91[b]	2.39[a]	1.80[b]	1.84[b]	0.088	0.03	<0.001	<0.001

[a,b,c] Within each row, values that do not share a common superscript are different (P≤0.05)

[1]Least squares means and the pooled standard error of the mean are presented; [2]ROI1: Republic of Ireland medium health; [3]ROI2: Republic of Ireland high health; [4]NI: Northern Ireland; [5]AT: Austria; [6]Pw: post-weaning; [7]ADFI: Average daily feed intake; [8]ADG: Average daily gain; [9]FCE: Feed conversion efficiency

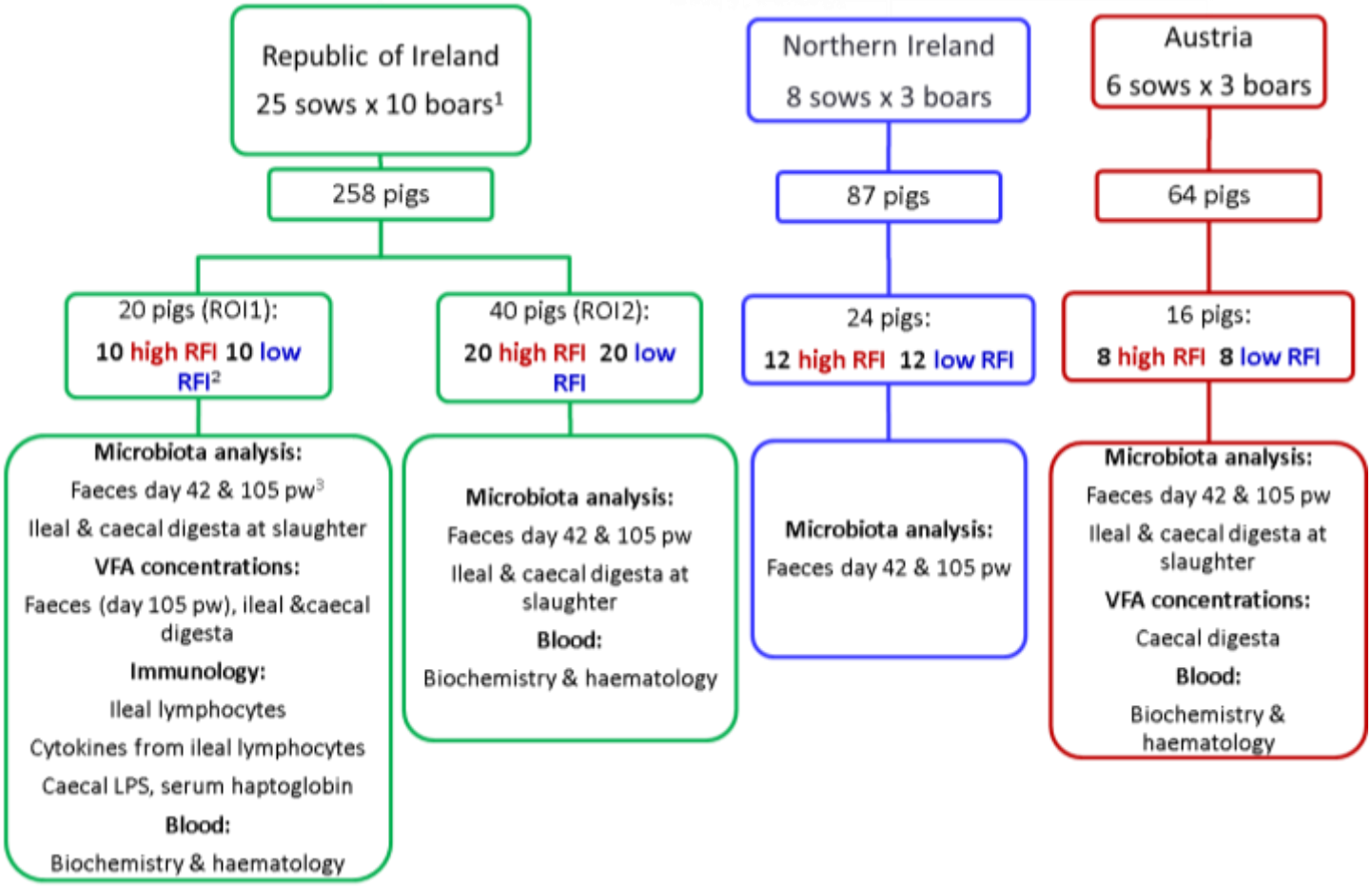


Figure 3.1. Schematic showing pig selection based on residual feed intake (RFI) and sampling across the geographical locations [1]One common boar was used across the three locations to minimise genetic variation; [2]Pigs were ranked on RFI between day 42 and 91 post-weaning; [3]Pw: post-weaning.

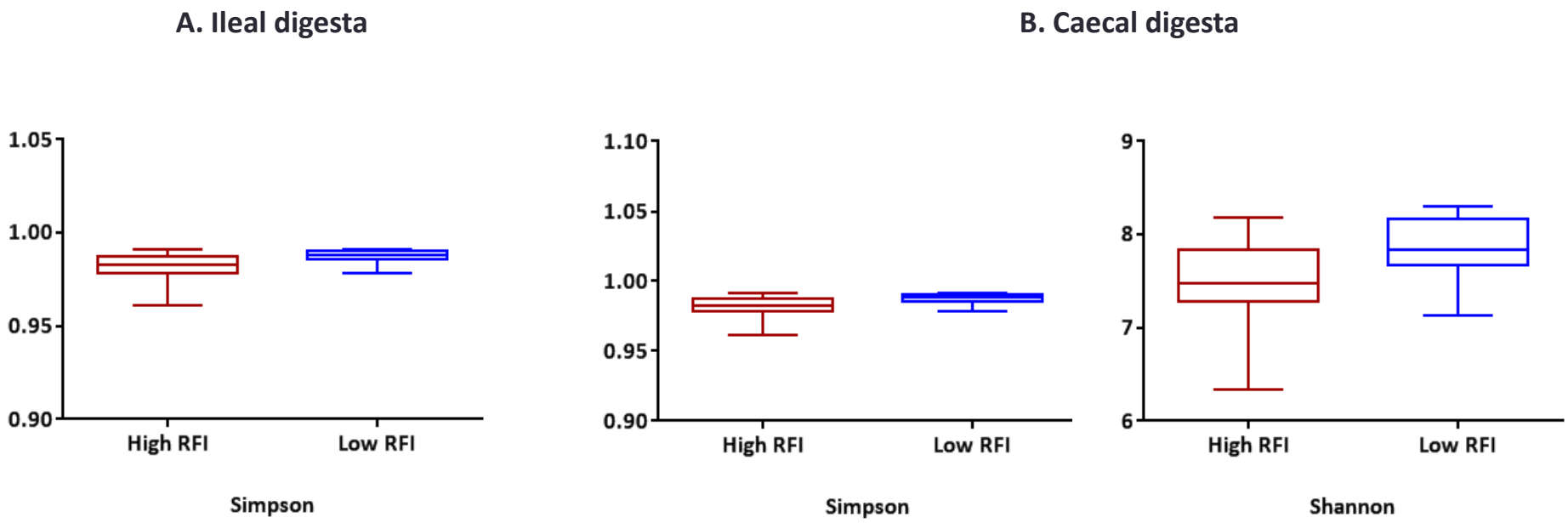


Figure 3.2. α-diversity of the intestinal microbiota of pigs ranked on residual feed intake (RFI) from ROI2 in A. Ileal and B. Caecal digesta (P<0.05)

Only the significant differences between high and low RFI rank for each index are shown.

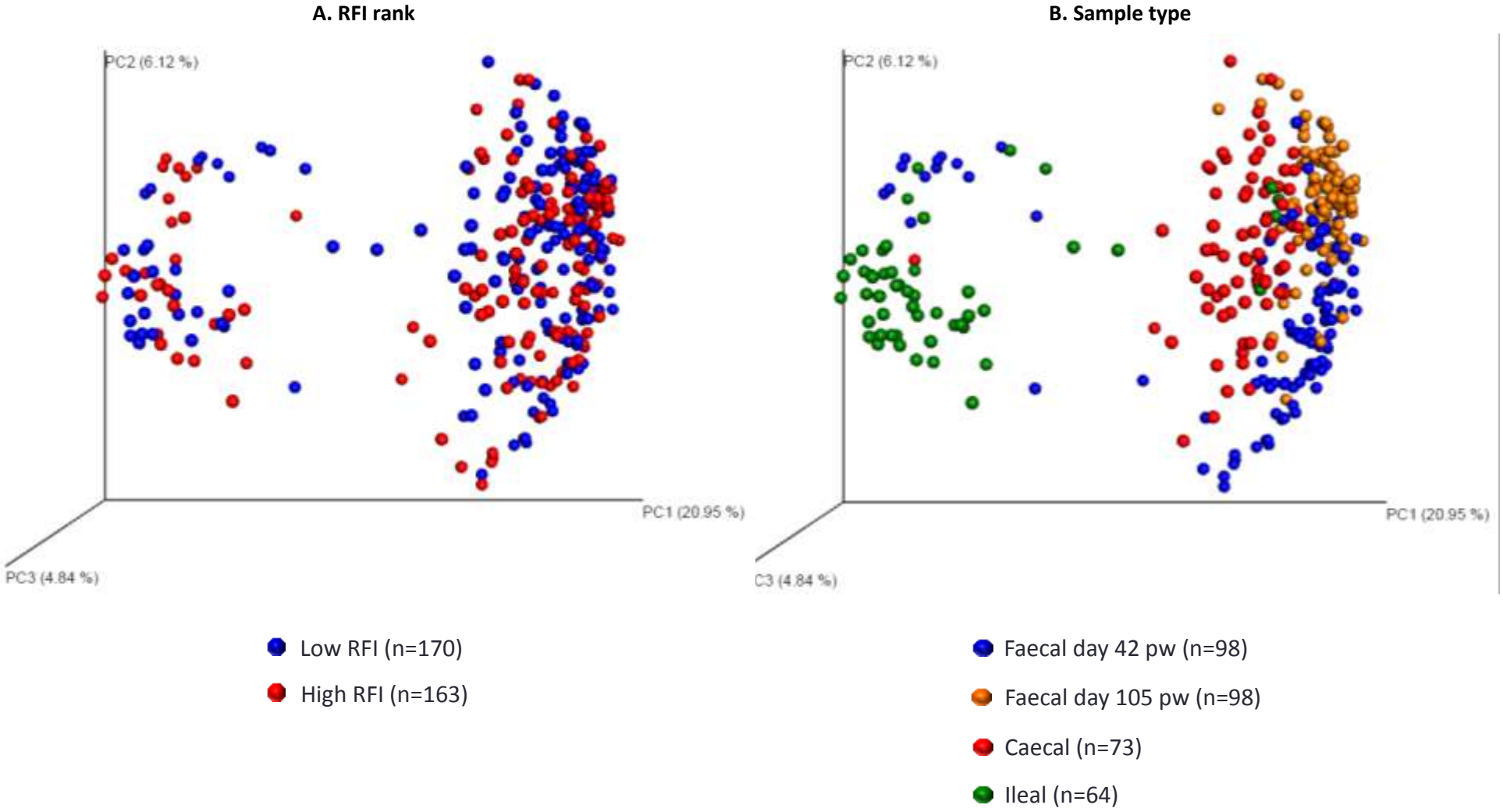


Figure 3.3. Principal coordinate analysis (PCoA) plots (based on OTUs) for all faecal and intestinal samples across geographical locations according to: A. RFI rank and B. Sample type

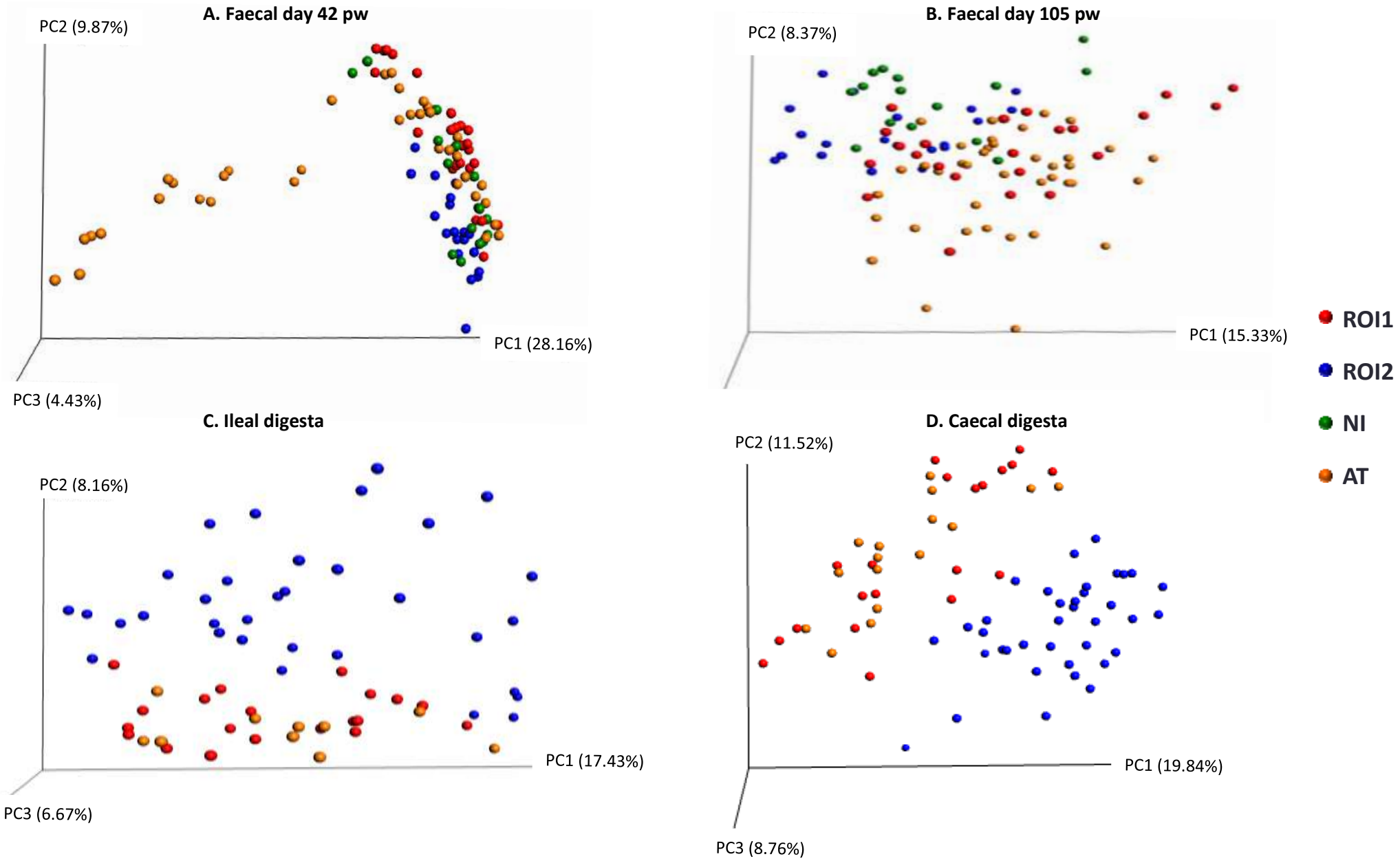
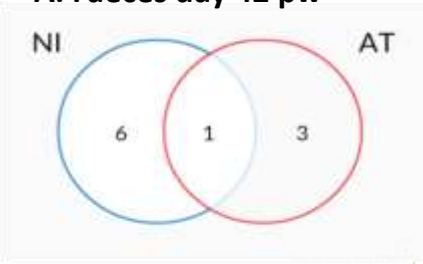
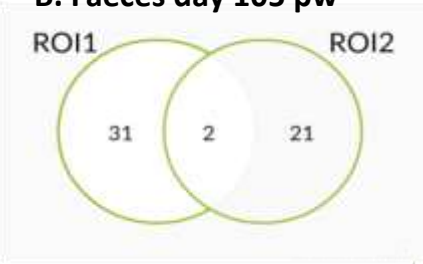

A. Faecal day 42 pw
PC2 (9.87%)
PC1 (28.16%)
PC3 (4.43%)
B. Faecal day 105 pw
PC2 (8.37%)
PC1 (15.33%)
C. Ileal digesta
PC2 (8.16%)
PC1 (17.43%)
PC3 (6.67%)
D. Caecal digesta
PC2 (11.52%)
PC1 (19.84%)
PC3 (8.76%)
ROI1
ROI2
NI
AT

Figure 3.4. Principal coordinate analysis (PCoA) plots (based on OTUs) for: A. Faeces collected on day 42 pw, B. Faeces collected on day 105 pw, C. Ileal digesta and D. Caecal digesta

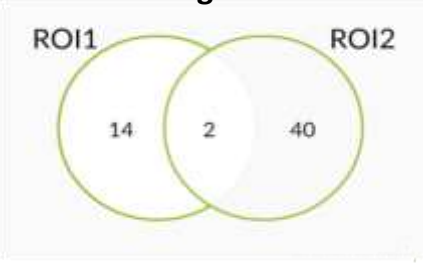
A. Faeces day 42 pw

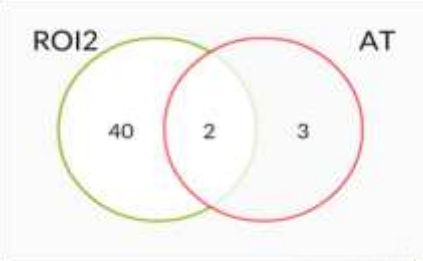


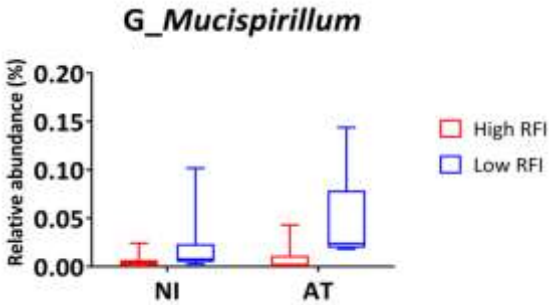
B. Faeces day 105 pw

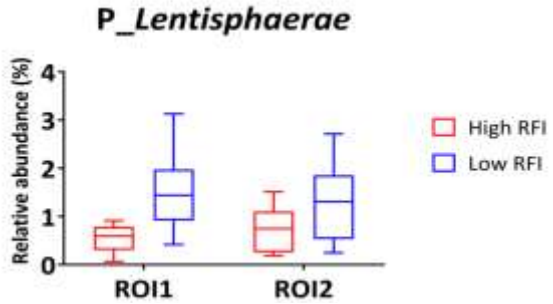


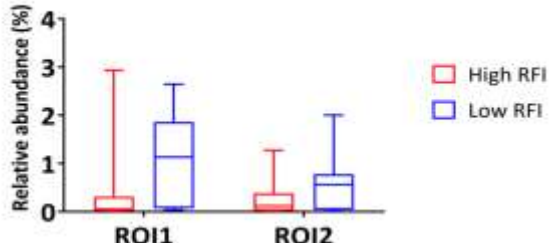
C. Caecal digesta

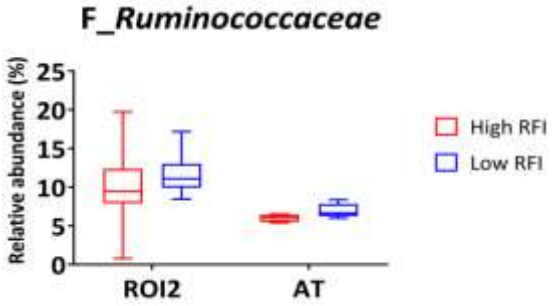


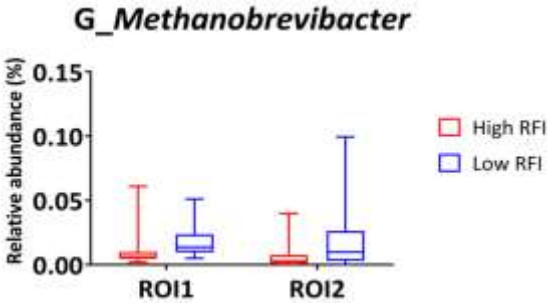


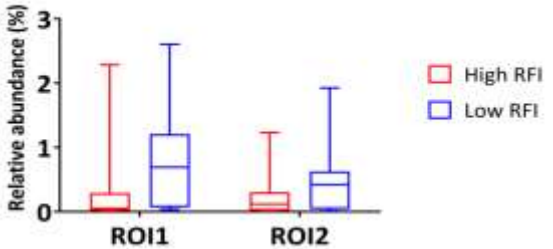












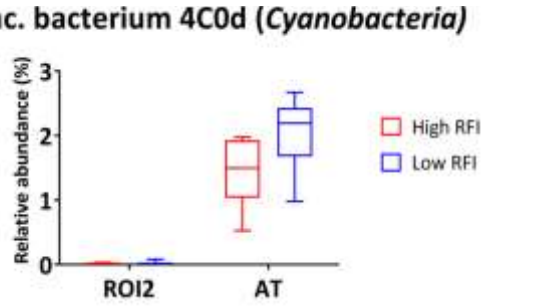


Figure 3.5. Commonality of RFI-associated bacterial taxa across geographical locations by sample type and relative abundance of the common taxa in both low and high RFI pigs (P<0.05)

Only those taxa that were significantly different between high and low RFI across locations are depicted.

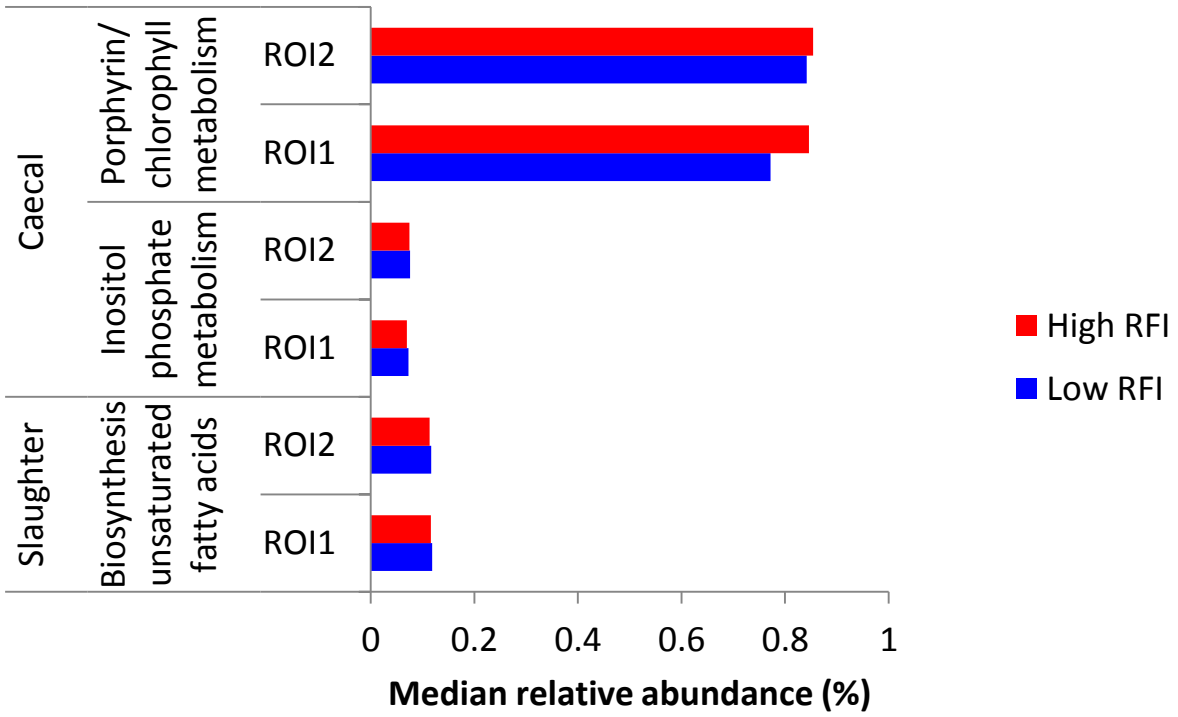


Figure 3.6. Median relative abundance (%) of common predicted microbial pathways differentially abundant in high and low RFI pigs (P<0.05) across geographical locations and by sample type

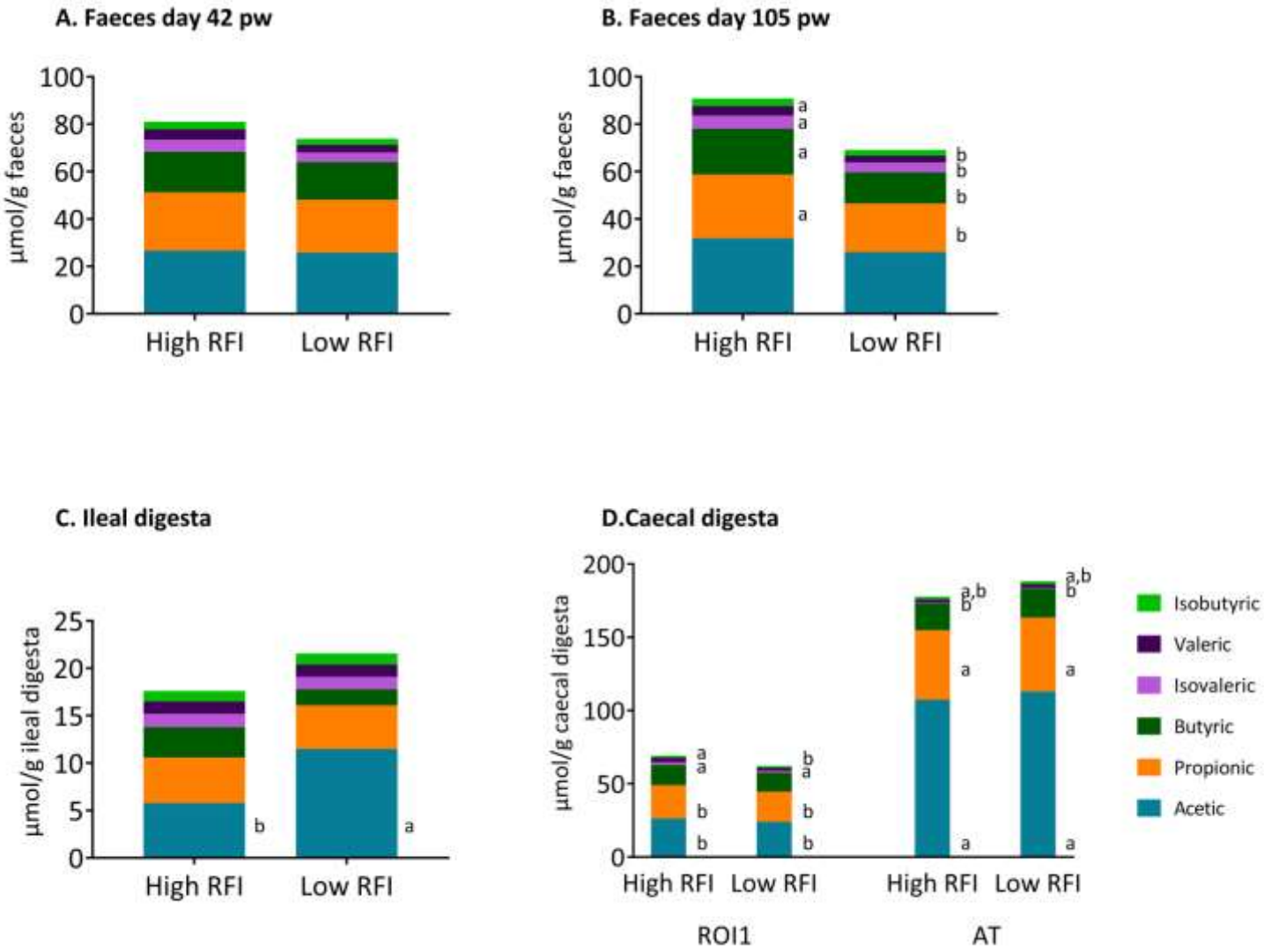

A. Faeces day 42 pw
µmol/g faeces
100
80
60
40
20
0
High RFI
Low RFI
B. Faeces day 105 pw
µmol/g faeces
a
a
a
a
b
b
b
b
High RFI
Low RFI
C. Ileal digesta
µmol/g ileal digesta
25
20
15
10
5
0
b
a
High RFI
Low RFI
D.Caecal digesta
µmol/g caecal digesta
200
150
100
50
0
a
a
b
b
b
a
b
b
a,b
b
a
a
a,b
b
a
a
High RFI
Low RFI
High RFI
Low RFI
ROI1
AT
Isobutyric
Valeric
Isovaleric
Butyric
Propionic
Acetic

Figure 3.7. Effect of ranking pigs on residual feed intake (RFI) on volatile fatty acid (VFA) concentrations (mmol/g) in faeces of ROI1 pigs at A. day 42 post-weaning, B. day 105 post-weaning, C. in the ileal digesta of ROI1 pigs and D. in the caecal digesta of ROI1 and AT pigs

ROI1: Republic of Ireland batch 1 pigs; AT: Austria batch of pigs.

[a,b,c] Within each volatile fatty acid, values that do not share a common superscript are significantly different ($P \leq 0.05$)

3.8. Supplementary Information

Table S3.1. Composition and chemical analysis of diets used in the study (on an as fed basis; g/kg)

Diet Type	Pig				Sow	
	Starter	Link	Weaner	Finisher	Gestation	Lactation
Barley			248.0	385.4	897.4	349.5
Wheat	220.0	399.0	431.4	404.0		432.4
Maize	80.0					
Soya	163.5	229.2	200.0	175.0	70.0	150.0
Full fat soya	100.0	70.0	50.0			
Lactofeed 70[1]	200.0	200.0				
Skim milk powder	125.0	50.0				
Soya oil	78.1	25.0	40.0	10.0	10.0	40.0
Lysine HCl (78.8)	4.73	3.70	4.6	4.0	1.0	3.5
DL-Methionine	3.22	2.33	1.7	1.0		1.0
L-Threonine (98)	2.41	1.62	2.0	1.5		1.0
L-Tryptophan	0.95	0.54	0.2	0.0		
Vitamin and mineral mix	3.0[2]	3.0[2]	3.0[2]	1.0[3]	1.5[4]	1.5[4]
Natuphos 5000 FTU/g[5]	0.10	0.10	0.1	0.1	0.1	0.1
Salt feed grade	3.00	3.00	3.0	3.0	4.0	4.0
Dicalcium phosphate	5.00	1.52	5.0	2.0	5.0	5.0
Limestone flour	11.00	11.0	11.0	13.0	11.0	12.0
Chemical analysis (g/kg dry matter)						
Crude protein	235.9	252.8	211.0	205.4	195.7	172.1
Crude fibre	19.8	23.4	34.8	37.5	38.9	31.7
Crude ash	66.2	63.5	48.3	45.4	44.6	49.8
Ether extract	114.7	57.9	70.7	27.2	33.2	63.4
Digestible energy (MJ/kg)[6]	17.9	17.0	16.9	16.0	15.9	16.3
Net energy (MJ/kg)[6]	11.4	10.3	10.6	9.8	9.5	10.5
Amino acids (g/kg)						
Lysine	16.2	15.0	13.0	11.1	6.4	9.9
Methionine	6.8	5.7	4.5	3.6	2.1	3.4
Methionine + cysteine	9.7	9.0	7.9	6.8	4.7	6.4
Threonine	10.5	9.8	8.7	7.5	4.5	6.5
Tryptophan	3.6	3.3	2.6	2.2	1.6	2.0

[1]Lactofeed 70 contains 70% lactose, 11.5% protein, 0.5% oil, 7.5% ash and 0.5% fibre (Volac, Cambridge, UK). [2]Premix provided per kg of complete diet: Cu, 155 mg; Fe, 90 mg; Mn, 47 mg; Zn, 120 mg, I, 0.6 mg; Se, 0.3 mg; vitamin A, 6000 IU; vitamin D_3, 1000 IU; vitamin E, 100 IU; vitamin K, 4 mg; vitamin B_{12}, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B_1, 2 mg; vitamin B_6, 3 mg; Endox, 60 g. [3]Premix provided per kg of complete diet: Cu, 15 mg; Fe, 24 mg; Mn, 31 mg; Zn, 80 mg, I, 0.3 mg; Se, 0.2 mg; vitamin A, 2000 IU; vitamin D_3, 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin B_{12}, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B_1, 2 mg; vitamin B_6, 3 mg. [4] Premix provided per kg of complete diet: Cu, 15 mg; Fe, 70 mg; Mn, 62 mg; Zn, 80 mg, I, 0.6 mg; Se, 0.2 mg; vitamin A, 1000 IU; vitamin D_3, 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; vitamin B_{12}, 15 µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; Biotin, 200 mg; Folic acid, 5 g; vitamin B_1, 2 mg; vitamin B_6, 3 mg. [5]Phytase; 5000 FTU/g equal to 500 FTU per kg finished feed.[6]Digestible energy and net energy were calculated from book values. Diets were pelleted to 3mm diameter after steam conditioning to 50-55 °C.

Table S3.2. Effect of residual feed intake (RFI) on salivary cortisol[1] and serum biochemistry parameters[2] for pigs from ROI and AT.

Parameter	High RFI	Low RFI	SEM[3]	P-value
Salivary cortisol (ng/ml)	1.76	1.34	0.176	0.06
Creatinine (µmol/L)	122	129	6.2	0.18
Creatine kinase (µmol/L)	105	109	11.2	0.78
Total protein (g/L)	66.1	66.7	0.89	0.71
Triglycerides (mmol/L)	0.50	0.48	0.036	0.58
Glucose (mmol/L)	5.44	5.68	0.209	0.23
Cholesterol (mmol/L)	2.37	2.39	0.114	0.86
Blood urea nitrogen (mg/dL)	14.9	14.1	1.09	0.58

[1]Salivary cortisol was measured in 20 pigs in ROI1 on day 100 pw.

[2]Blood serum was collected from ROI1, ROI2 and AT pigs at slaughter (day 105 pw).

[3]Least squares means and the pooled standard error of the mean are presented.

Table S3.3. Effect of ranking pigs on residual feed intake (RFI) on haematological parameters in ROI and AT pigs

Parameter	High RFI	Low RFI	SEM[1]	P-value
White blood cells (x 10^3 cells/µl)	23.0	23.2	1.56	0.93
Lymphocytes				
%	44.0	47.4	3.07	0.23
no. x 10^3 cells/µl	9.6	10.4	0.95	0.39
Monocytes				
%	12.0	10.6	1.70	0.30
no. x 10^3 cells/µl	2.97	2.59	0.384	0.25
Granulocytes				
%	40.2	41.1	3.95	0.87
no. x 10^3 cells/µl	10.3	9.9	1.31	0.81
Red blood cells (x 10^6 cells/µL)	6.59	6.63	0.301	0.90
Red cell distribution width (fL)	19.8	20.1	0.99	0.73
Haemoglobin (g/dL)	12.7	12.6	0.39	0.70
Haematocrit (%)	0.37	0.37	0.011	0.97
Mean corpuscular volume (fL)	53.8	55.0	0.78	0.21
Mean corpuscular haemoglobin				
%	31.2	31.7	0.46	0.29
pg	17.2	17.3	0.26	0.76
Platelets (x 10^6 cells /µL)	219.8	196.7	23.6	0.38
Mean platelet volume (fL)	8.93	8.70	0.490	0.72

[1]Least squares means and the pooled standard error of the mean are presented.

Table S3.4. Pooled ileal intraepithelial (IEL) and lamina propria (LPL) lymphocyte populations (%)[1], with or without mitogen stimulation, from ROI1 pigs ranked on residual feed intake (RFI) and cytokine production (pg/ml) from these cells

	High RFI	Low RFI	SEM[2]	P-value
Pooled ileal LPL and IEL cells				
Control (PBS)				
B cells	1.74	1.79	0.484	0.99
T cells	2.49	1.82	0.589	0.86
CD4 T cells	80.9	87.5	8.39	0.95
CD8 T cells	6.36	5.81	1.129	0.98
CD4CD8 T cells	6.26	6.41	2.433	0.99
γδ T cells	0.97	0.82	0.156	0.91
Monocytes	7.94	7.66	2.282	0.99
Mitogen-stimulated (PMA+I)[3]				
B cells	1.86	2.04	0.485	0.99
T cells	3.86	2.52	0.590	0.39
CD4 T cells	70.3	81.9	8.40	0.76
CD8 T cells	5.71	4.79	1.130	0.93
CD4CD8 T cells	4.78	11.02	2.432	0.18
γδ T cells	0.62	0.64	0.157	0.99
Monocytes	6.77	4.47	2.282	0.89
Cytokine production from pooled ileal LPL and IEL				
Control (PBS)				
IL-4	3.28	4.94	0.260	0.69
IL-6	3.77	2.41	0.310	0.76
IL-8	7.76	5.61	0.403	0.61
TNFα	3.28	3.92	0.302	0.98
Mitogen-stimulated (PMA+I)				
IL-4	4.16	3.86	0.270	0.97
IL-6	4.36	4.08	0.320	0.99
IL-8	5.93	6.33	0.400	0.71
TNFα	2.58	3.73	0.300	0.83

[1]Percentages are based on total white blood cells.

[2]Least squares means and the pooled standard error of the mean are presented.

[3]PMA+I: pooled IEL and LPL were mitogen-stimulated with phorbol myristate acetate (PMA, 25 ng/mL) plus ionomycin (I; 1 μg/mL).

Table S3.5. Caecal lipopolysaccharides[1] and serum haptoglobin concentrations[2] in pigs ranked on residual feed intake (RFI) across geographical locations

Measure	High RFI				Low RFI				SEM[3]	P-value
	ROI1	ROI2	NI	AT	ROI1	ROI2	NI	AT		
Log EU[4]/g FM[5]	6.15	6.09	6.07	6.16	6.17	6.01	6.02	6.11	0.065	0.76
Log EU/g DM[5]	7.03	6.97	7.05	7.11	7.06	6.92	7.05	7.10	0.062	0.86
Serum haptoglobin (µg/ml)	172.1	-	171.4	180.8	155.8	-	186.6	180.4	35.74	0.47

[1]Caecal lipopolysaccharides were measured in digesta collected at slaughter (day 105 post-weaning); [2]Haptoglobin concentrations were measured in serum collected (from ROI1, AT and NI pigs).

[3]Least squares means and the pooled standard error of the mean are presented.

[4]EU: endotoxin units; [5]FM: fresh matter; [6]DM: dry matter.

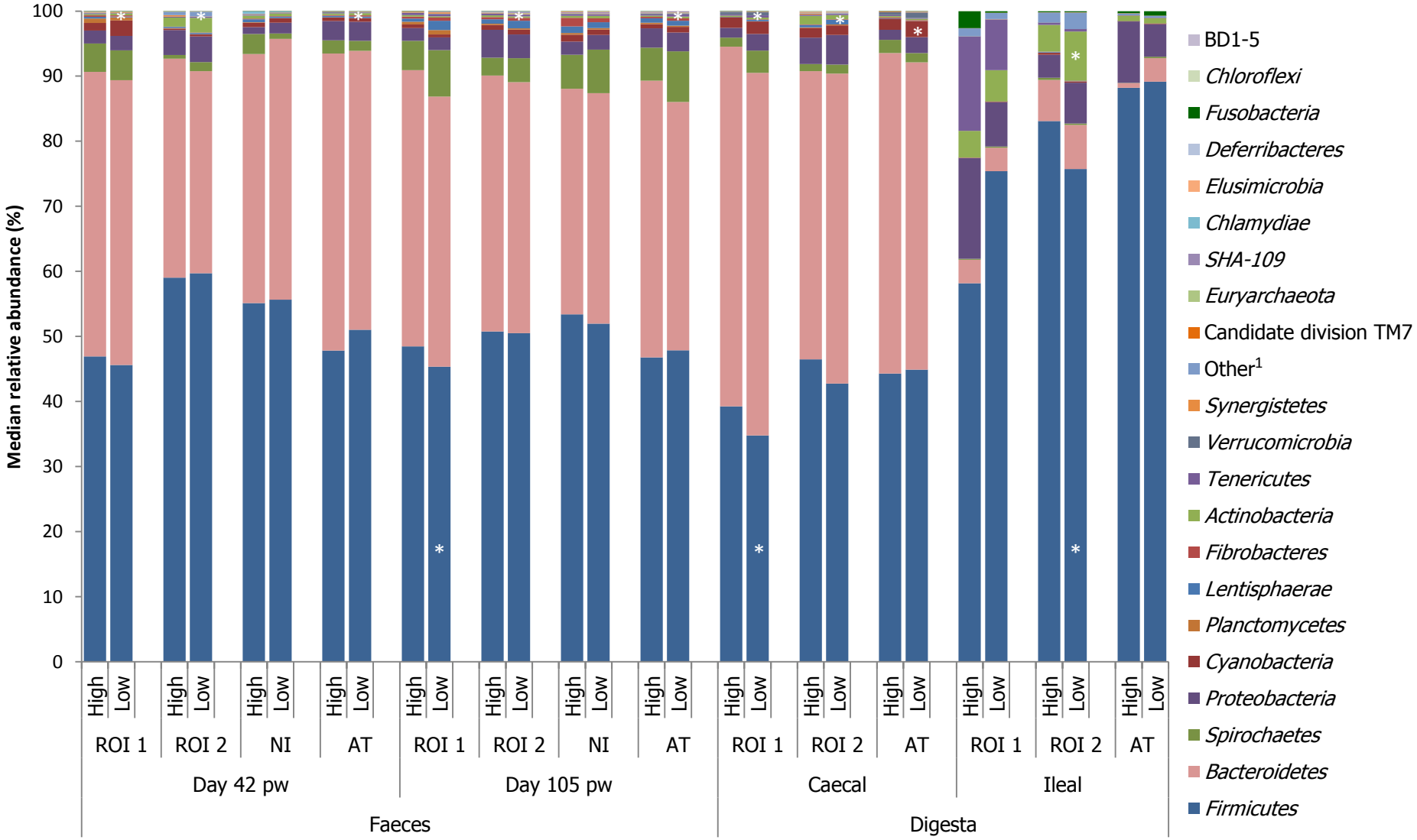

Median relative abundance (%)
100
90
80
70
60
50
40
30
20
10
0
High
Low
ROI 1
ROI 2
NI
AT
Day 42 pw
Day 105 pw
Caecal
Ileal
Faeces
Digesta
BD1-5
Chloroflexi
Fusobacteria
Deferribacteres
Elusimicrobia
Chlamydiae
SHA-109
Euryarchaeota
Candidate division TM7
Other[1]
Synergistetes
Verrucomicrobia
Tenericutes
Actinobacteria
Fibrobacteres
Lentisphaerae
Planctomycetes
Cyanobacteria
Proteobacteria
Spirochaetes
Bacteroidetes
Firmicutes

Figure S3.1. Median relative abundance (%) of bacterial phyla present in pigs according to RFI rank across geographic locations for all faecal time points and intestinal sites

[1] No blast hits/uncultured. *Indicate significant differences observed between high and low RFI pigs within each sample type and geographic location at P≤0.05.

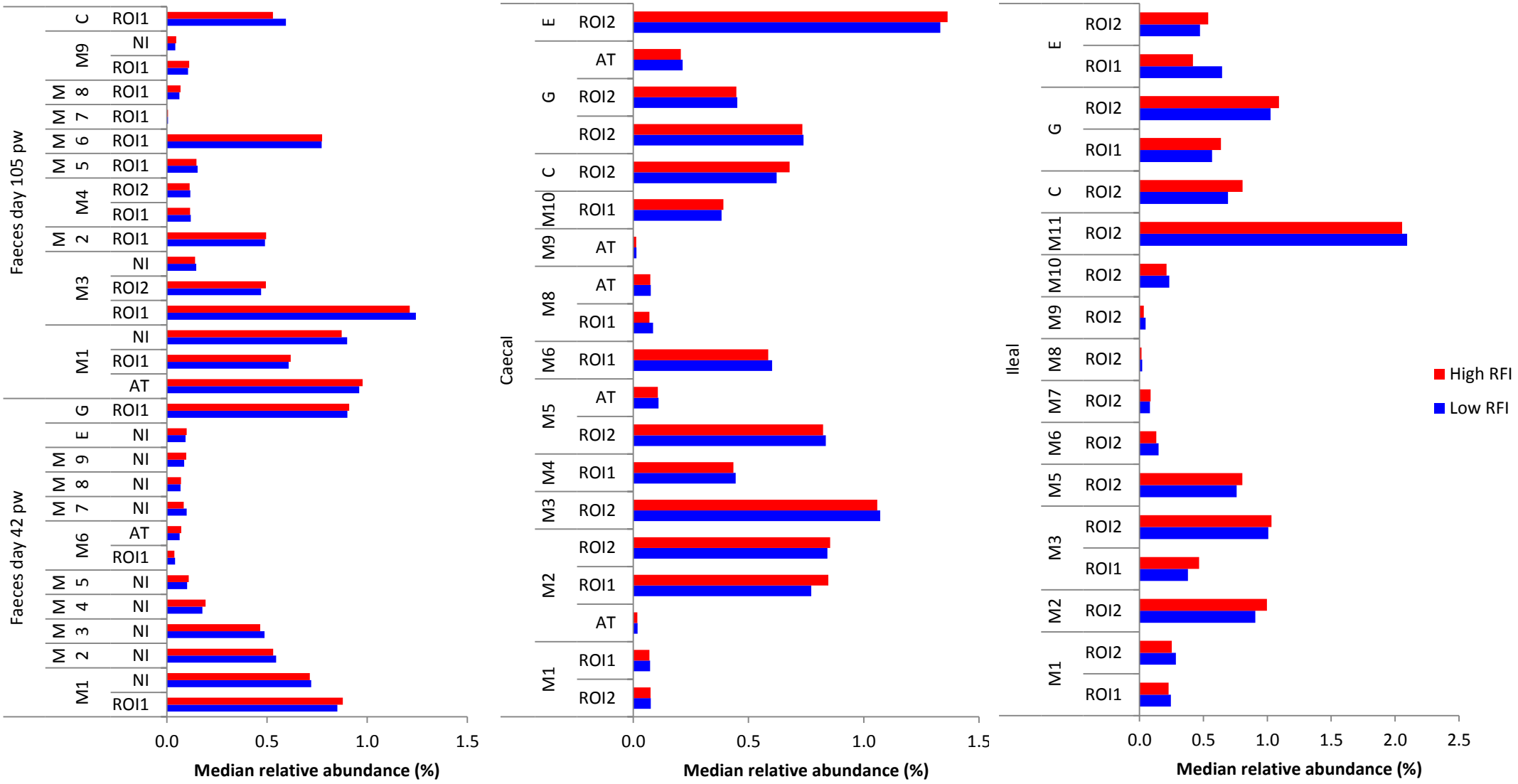

Faeces day 105 pw
C ROI1
M9 NI
M9 ROI1
M 8 ROI1
M 7 ROI1
M 6 ROI1
M 5 ROI1
M4 ROI2
M4 ROI1
M 2 ROI1
M3 NI
M3 ROI2
M3 ROI1
M1 NI
M1 ROI1
M1 AT
Faeces day 42 pw
G ROI1
E NI
M 9 NI
M 8 NI
M 7 NI
M6 AT
M6 ROI1
M 5 NI
M 4 NI
M 3 NI
M 2 NI
M1 NI
M1 ROI1
0.0 0.5 1.0 1.5
Median relative abundance (%)
Caecal
E ROI2
G AT
G ROI2
ROI2
C ROI2
M10 ROI1
M9 AT
M8 AT
M8 ROI1
M6 ROI1
M5 AT
M5 ROI2
M4 ROI1
M3 ROI2
M2 ROI2
M2 ROI1
M2 AT
M1 ROI1
M1 ROI2
0.0 0.5 1.0 1.5
Median relative abundance (%)
Ileal
E ROI2
E ROI1
G ROI2
G ROI1
C ROI2
M11 ROI2
M10 ROI2
M9 ROI2
M8 ROI2
M7 ROI2
M6 ROI2
M5 ROI2
M3 ROI2
M3 ROI1
M2 ROI2
M1 ROI2
M1 ROI1
0.0 0.5 1.0 1.5 2.0 2.5
Median relative abundance (%)
High RFI
Low RFI

Figure S3.2. Predicted functionality of intestinal microbiota for high and low RFI pigs across geographic locations and by sample type A. Faeces, B. Caecum, C. Ileum. Pathways shown are those found to be differentially abundant between high and low RFI pigs (P<0.05)

Pathways were grouped into major functional categories as follows: M1: Carbohydrate metabolism, M2: Metabolism of cofactors and vitamins, M3: Energy metabolism, M4: Lipid metabolism, M5: Amino acid metabolism, M6: Glycan biosynthesis and metabolism, M7: Biosynthesis of other secondary metabolites, M8: Metabolism of terpenoids and polyketides, M9: Xenobiotic biodegradation and metabolism, M10: Metabolism of other amino acids, M11: Nucleotide metabolism, E: Environmental information processing, G: Genetic information processing, C: Cell motility.

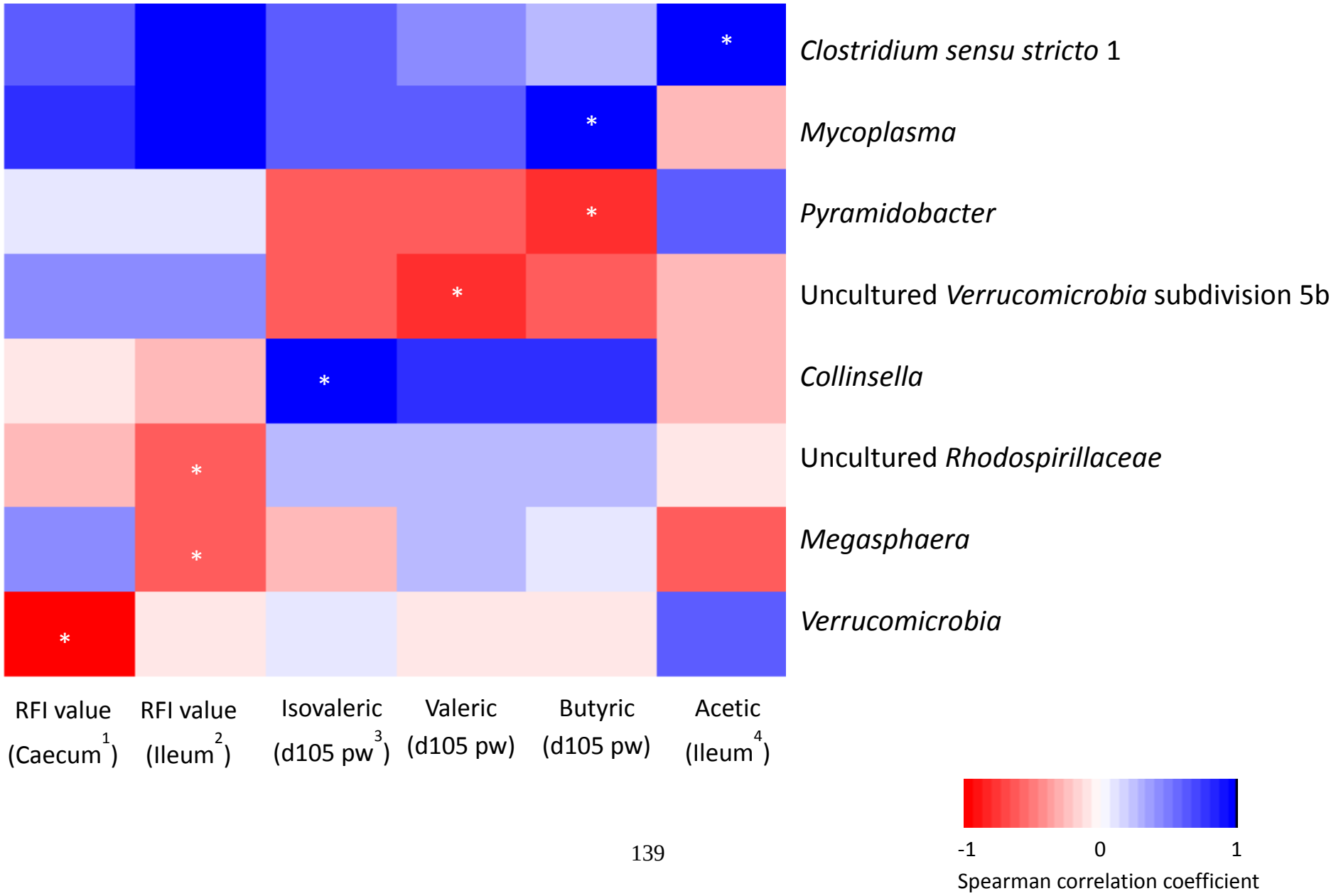

*
*
*
*
*
*
*
*
Clostridium sensu stricto 1
Mycoplasma
Pyramidobacter
Uncultured Verrucomicrobia subdivision 5b
Collinsella
Uncultured Rhodospirillaceae
Megasphaera
Verrucomicrobia
RFI value (Caecum[1])
RFI value (Ileum[2])
Isovaleric (d105 pw[3])
Valeric (d105 pw)
Butyric (d105 pw)
Acetic (Ileum[4])
-1
0
1
Spearman correlation coefficient

Figure S3.3. Heat map showing Spearman correlations between bacterial taxa and physiological measures in pigs ranked by residual feed intake (RFI)
Correlations were only performed between bacterial taxa at the phylum and the genus levels with RFI value and volatile fatty acids that differed significantly between high and low RFI pigs. *Indicates significance at P≤0.05. Red indicates negative correlation and blue indicates positive correlation. [1]Correlated with the intestinal microbiota in the caecum of pigs from the Republic of Ireland (ROI; batch 1); [2]Correlated with the intestinal microiota.in the ileum of pigs from ROI (batch 2); [3]Correlated with the faecal microbiota in the faeces of pigs collected on day 105 post-weaning (pw) from ROI (batch 1); [4]Correlated with the intestinal microbiota in the ileum of pigs from ROI (batch 1).

4. Faecal microbiota transplantation in sows and/or neonatal offspring alters lifetime intestinal microbiota and growth in offspring

UM. McCormack, T. Curião, T. Wilkinson, BU. Metzler-Zebeli, H. Reyer, JA. Calderon-Diaz, T. Ryan, F. Crispie, PD. Cotter, CJ. Creevey, GE. Gardiner, PG. Lawlor. 2017. Faecal microbiota transplantation in sows and/or neonatal offspring alters lifetime intestinal microbiota and growth in offspring.

(Submitted to *mSystems* September 2017)

4.1 Abstract

Previous studies suggest a link between the intestinal microbiota and feed efficiency (FE) in pigs. Therefore, we investigated if faecal microbiota transplantation (FMT) in sows and/or offspring, using inocula derived from highly feed efficient pigs, could improve offspring FE. Pregnant sows were assigned to control or FMT treatments, and the resultant offspring to control, FMT once, or FMT four times. The FMT altered sow faecal and colostrum microbial profiles, and resulted in lighter body weight in 70- and 155-day old offspring. This was accompanied by FMT-associated changes within the offspring intestinal microbiota, including transiently higher faecal bacterial diversity and load, and numerous compositional differences at the phylum (e.g. *Spirochaetes* and *Bacteroidetes* at high relative abundance) and genus levels (mostly *Firmicutes* members), as well as differences in abundance of predicted bacterial pathways, all mostly within the ileal microbiota. Intestinal morphology was negatively impacted, duodenal gene expression altered, and serum protein and cholesterol concentrations reduced due to FMT. Taken together, results suggest poorer absorptive capacity and intestinal health, possibly explaining the reduced body weight. Although these findings have negative implications for the use of FMT for the improvement of FE in pigs, they nonetheless demonstrate the impact of early life intestinal microbiota.

4.2. Introduction

The porcine intestinal microbiome is considered an important 'organ', playing a major role in nutrient metabolism and immune response (Kim and Isaacson, 2015a, Kim et al., 2015b, Xiao et al., 2016, Zhao et al., 2015). Recently, intestinal microbiota composition has been linked with growth in pigs (Ramayo-Caldas et al., 2016, Mach et al., 2015, Yang et al., 2016). Moreover, previous work from Chapters 2 and 3 showed that microbial composition and predicted functionality are associated with porcine feed efficiency (FE). As FE is a major determinant of profitability in pig production, strategies to improve FE are continuously being sought. To date, several approaches have been applied to increase beneficial gut bacterial populations with a view to improving FE including probiotics (Prieto et al., 2014, Zimmermann et al., 2016), prebiotics (Grela et al., 2014, O'Sullivan et al., 2010, de Lange et al., 2010) and synbiotics (Lee et al., 2009, Sattler et al., 2015). Here, for the first time, we attempt to improve FE through faecal microbiota transplantation (FMT) to pregnant sows and their offspring.

Faecal microbiota transplantation involves the transfer of donor microbiome (i.e. faecal material), to a recipient in order to establish a more desirable microbiome. The aim is to populate the gastrointestinal tract (GIT) with potentially beneficial bacteria, thereby establishing/restoring intestinal homeostasis (Nieuwdorp, 2014, Xu et al., 2015). To date, FMT has been exploited in humans to treat recurrent *Clostridium difficile* infection (Borody et al., 2014). It is also under investigation for treatment of enteric infections and inflammatory bowel disorders (Nieuwdorp, 2014), as well as metabolic and autoimmune diseases (Xu et al., 2015). One of the main advantages of FMT is that it provides a full range of microbiota (Borody et al., 2014). However, there are limitations regarding collection, preparation and storage of donor faeces (Kelly, 2015), as well as selection of suitable donors (Vermeire et al., 2016).

As regards pigs, FMT has been used to generate a human microbiota-associated pig model (Pang, 2007, Zhang, 2013), and tograft porcine microbiota into the rodent GIT (Hirayama, 1999, Yan et al., 2016). Interestingly, FMT from three pig breeds resulted in similar intestinal structure, gene expression and enzymatic activities in germ-free mice (Diao et al., 2016). However, to our knowledge, only only a limited number of studies have conducted pig-to-pig microbiota transfer. In one (conference abstract), FMT to piglets was successful in preventing necrotising entero-colitis in a piglet model of the disease, but increased neonatal mortality (Martin et al., 2015). In another study, immunologic characteristics were transferred from donor to recipient as a result of microbiota transplantation from on pig breed to another (Xiao et al., 2017). These studies provide evidence of the ability to 'reprogramme' the porcine intestinal microbiota via FMT with resultant alterations in host phenotype. However, the use of FMT as a tool to improve FE in pigs has yet to be investigated. Therefore, the purpose of this study was to investigate if oral FMT with faecal extracts from highly feed efficient pigs, in sows and/or their offspring, would improve FE via beneficial modulation of the intestinal microbiota, and if so, to determine if the effect(s) persists throughout the lifetime of the offspring.

4.3. Materials and methods

4.3.1. Ethical approval

The pig study was approved by the Teagasc and Waterford Institute of Technology animal ethics committees and performed according to European Union regulations (91/630/EEC; 98/58/EC). An experimental license (AE1932/P032) was obtained from the Irish Health Products Regulatory Authority (HPRA). Piglets that died/were removed during the study are listed in Table S4.1.

4.3.2. Faecal microbiota transplantation in sows

A schematic showing preparation of the FMT inoculum from the faeces of highly feed efficient donor pigs (from Chapter 3) is shown in Figure 4.1 and an overview of the sow and offspring treatments, and sample collection in Figure S4.1. At day 60 of gestation, sows were blocked by boar and body weight and assigned to one of two treatments: 1) Control (CON; n=9) and 2) FMT procedure (FMTP; n=9). The FMTP refers to all steps used in the procedure (the details of which are outlined below) i.e. antibiotic treatment, purgative, fasting, proton-pump inhibitor and both FMTs.

On day 61, sows on the FMT treatment were individually housed and commenced a 7-day course of antibiotics (cocktail of three antibiotics used for its broad spectrum of activity top-dressed on feed) (Figure S4.1). To ensure that the FMT was not inactivated by the antibiotics, 3 days elapsed between the last dose of antibiotic and the first FMT.

On day 68, FMT sows received a purgative (Figure S4.1) in two doses (one in the morning and one in the evening) via gastric intubation, and were fasted from the first dose of the purgative until day 70 of gestation, when they received a proton-pump inhibitor (Figure S4.1) 1h prior to FMT. Aliquots (100 mL) of the FMT inoculum containing a mean count of 1.4. x 10^9 CFU/mL as determined by plating on Wilkins

Chalgren (WC) anaerobe agar (Oxoid Ltd, Basingstoke, Hampshire, UK), were thawed to room temperature and administered via gastric intubation to each sow in the FMT group [200 mL/sow; which delivered a dose of 2.8 x 10^{11} CFU, referred to as 'first FMT']. The gastric tube was then rinsed with a solution of omeprazole (120 mg/sow) dissolved in 400 mL lukewarm water.

On day 100, FMT sows received the 'second FMT' (containing a mean count of 1.2. x 10^{9} CFU/mL, as determined by plating on WC anaerobe agar, which delivered a dose of 2.5 x 10^{11} CFU), using the same procedure as on day 70. Faecal samples for microbiota analysis were collected throughout gestation from 6 sows/treatment, as outlined in Figure1A, snap-frozen in liquid nitrogen and stored at -80 °C. At the onset of farrowing, the two teats immediately distal to the sows head were cleaned with iodine and colostrum was manually collected (from 6 sows/treatment) into sterile containers and stored at -80 °C for microbiota analysis.

4.3.3. Faecal microbiota transplantation, sampling and management of offspring

At farrowing, nine piglets (n=162) were selected as having average birth weights for their respective litter, blocked by gender and weight, and randomly assigned to one of three treatments: 1) Control (CON), 2) FMT at birth (FMT1), and 3) FMT at birth and days 3, 7 and 28 of age (FMT4) (Figure S4.1). This resulted in 6 treatments (sow x offspring treatment interaction): CON/CON, CON/FMT1, CON/FMT4 and FMT/CON, FMT/FMT1 and FMT/FMT4. The FMT in piglets was performed by orally administering (via syringe) 8 mL of inoculum containing a mean count of 1.2 x 10^{9} CFU/mL (as determined on WC anaerobe agar), which delivered a dose of 9.6 x 10^{9} CFU.

At weaning, six pigs per litter (2/offspring treatment; one male and one female where possible) were selected from 7 litters per sow treatment (n=42 pigs/sow

treatment; n=28 pigs/offspring treatment; Figure S4.1). All pigs were individually housed from weaning to slaughter (details given in Figure S4.1) and fed a common sequence of the same diets (Table S4.2). Body weight and feed disappearance were recorded weekly to calculate performance (ADFI, ADG, FCE, RFI).

Throughout the study, faeces were collected from 36 pigs [1 pig/treatment/litter (same gender per litter) from six litters per sow treatment] by rectal stimulation at four time points for microbiota analysis (Figure S4.1). Intact litters were selected to control for inter-litter and inter-sow variation.

At ~155 days of age, all pigs were slaughtered by CO_2 stunning followed by exsanguination. Hot carcass weight was recorded immediately and cold carcass weight and kill-out percentage calculated and back-fat and muscle depth were recorded and used to estimate lean meat yield as described in Chapter 2. For microbiota and VFA analyses, ileal and caecal digesta was collected as detailed in Chapter 2, from the 36 selected pigs, and colon digesta was sampled 1 m distal to the caecum. Intestinal tissue was sampled from the duodenum, jejunum and ileum for histological analysis (Walsh et al., 2012). Mucosal scrapings were collected from 10 cm of duodenum tissue (5 cm distal to the location of the histological sample) using a glass slide, for gene expression and brush border enzyme activity analyses. All samples, except those for histological analysis, were snap frozen in liquid nitrogen and stored at -80°C until processing.

Blood was collected during exsanguination for haematology and biochemistry analyses from the 36 selected pigs (Figure S4.1) using vacuette tubes (Labstock, Dublin, Ireland) as described previously (Prieto et al., 2014).

4.3.4. Microbiota analysis in inocula, faecal, digesta and colostrum samples

Total DNA was extracted from all samples collected throughout the study from the inoculum (donors, aliquoted and thawed inocula), sows and offspring, using the

QIAamp DNA stool minikit (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions, apart from adding a beat beating step, and increasing the lysis temperature to 95°C (Buzoianu et al., 2012a). Total DNA was extracted from colostrum using the Powerfood microbial DNA isolation kit (Cambio Ltd, Cambridge, England) according to the manufacturer's instructions.

The V3-V4 region of the 16S rRNA gene (~ 460 bp) was sequenced (2 x 250 bp) using the Illumina MiSeq, following standard protocol as in Chapter 2. Sequence reads were checked for quality using FastQC software and adapters were removed (Illumina CLIP software). Reads were then trimmed to 240 bp at the end of the sequence using Trimommatic version 0.36 (Bolger et al., 2014), forward and reverse reads were merged using Flash (Magoc et al., 2011) and quality checks performed to guarantee maximum coverage. Reads were then clustered into operational taxonomical units (OTUs) using a 97% sequence identity threshold and chimeras removed with the CD-HIT-OTU pipeline. The Ribosomal Database Project classifier (RDP) was used (Wang et al., 2007) for taxonomic assignment, with a cut-off of 80%, with those <80% labelled as unclassified. Samples with <5,000 total joined reads were excluded from the analyses, except for colostrum samples, for which a cut-off of >1000 reads was applied. The OTU data were scaled to the minimum number of total reads for each sample type and filtered to remove OTUs at <100 reads. Alpha-diversity [Chao1 (OTU richness), Shannon and Simpson (OTU richness and abundance)] and beta-dispersion estimates were calculated using the Adonis2 and beta permutation functions in the Vegan package in R, each with 999 permutations. Principal component analysis (PCA) plots were generated with the bioconductor package DESeq2 (Love et al., 2014) and ggplot in R. Heatmaps of relative abundance were generated in GraphPad Prism 7.

Quantification of the 16S rRNA gene was performed in triplicate for all inocula, faecal and digesta samples by quantitative PCR (qPCR) in order to determine the total bacterial load, as described in Chapter 2.

4.3.5. Prediction of microbial function

The functionality of the microbiota for each sample based on 16S rRNA data and the 13_5 version of the Greengenes database for taxonomy and OTU assignments was predicted *in silico* using PICRUSt. Prediction of functions was inferred based on Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations. Pathways not related to bacteria, not relevant to porcine studies and for which the relative abundance in samples was <0.001% were dismissed.

4.3.6. Volatile fatty acid analysis and pH of digesta samples

Volatile fatty acid concentrations were measured in ileal, caecal and colonic digesta samples in triplicate using gas chromatography, as detailed in Chapter 2. Briefly, ~8 g of sample was weighed and pH-recorded, and a trichloroacetic acid (TCA) extraction performed. Extracts were mixed with an internal standard and 1 µL volumes injected into the gas chromatograph (Agilent 5890) under the following conditions; hydrogen (30 psi), helium (50 psi), and temperatures of 80 °C (oven), 280 °C (detector), and 250 °C (injector).

4.3.7. Intestinal histology

Tissue (~3 cm sections) collected from the duodenum, jejunum and ileum at slaughter was processed for histological analysis as in Chapter 2. Ten villi were examined per slide for villus height and width, crypt depth and goblet cell number using a light microscope at ×400 magnification.

4.3.8. Candidate gene expression and brush border enzyme activity in the duodenum

Total RNA was isolated from 20 mg duodenal mucosal scrapings using mechanical homogenization and the RNeasy Mini Kit (Qiagen, Hilden, Germany). Samples were homogenized using the FastPrep-24 instrument (MP Biomedicals, Santa Ana, CA, USA). Genomic DNA was removed and the RNA was quantified and evaluated and complementary DNA synthesized as previously outlined (Metzler-Zebeli et al., 2017a). The candidate genes measured by qPCR and the primers used are listed in Table S4.3. Amplifications were performed in 20 µL reactions on a real-time PCR Mx3000P thermocycler (Agilent Technologies, Waghaeusel-Wiesental, Germany) in duplicate, as described previously (Metzler-Zebeli et al., 2017a).

Duodenal maltase (EC 3.2.1.20), saccharase (EC3.2.1.48) and lactase (EC 3.2.1.23) activities [expressed as micromoles of substrate hydrolysed per minute per gram of protein (U/g protein)] were analysed as described previously (Martin et al., 2013).

4.3.9. Haematology and blood biochemistry analyses

Haematological analysis was performed using a Beckman Coulter Ac T Diff analyzer (Beckman Coulter Ltd., High Wycombe, UK). Total protein, blood urea nitrogen, cholesterol, glucose, triglycerides, creatinine and creatine kinase concentrations were determined in serum samples using an ABS Pentra 400 clinical chemistry analyser (Horiba, ABX, North Hampton, UK), calibrated according to the manufacturer's instructions, with every fifth sample run in duplicate to determine analyser accuracy.

4.3.10. Statistical analysis

All data were statistically analysed using SAS 9.3, using gender, treatment, boar and time point, where appropriate (weekly measures of growth performance), as fixed effects. Pig nested within sow was used as a random effect. The qPCR data were $\log_{10}$-transformed prior to statistical analysis, which was performed using a generalised linear mixed model (PROC GLIMMIX).

Microbial composition and predicted functionality data were analysed using generalised linear mixed model equation methods in PROC GLIMMIX. A gamma distribution was assumed for all data. Models for sow faeces and colostrum included sow treatment, faecal time point (if applicable) and their interactions as fixed effects. Offspring models included sow treatment, offspring treatment, faecal time point and their interactions as fixed effects. Additionally, a random intercept for each time point was included (repeated measure). A similar model was used for digesta collected at slaughter but intestinal site was included instead of faecal time point. In all models, data were back-transformed to the original distribution using the *ilink* option. Spearman rank-order correlations were performed between taxonomic relative abundances at the genus level bacterial genera, for each sample type and according to sow/offspring treatment, with body weight at slaughter, using the PROC CORR procedure, using the Stepdown Bonferroni test to correct for multiple comparisons. Heatmaps were produced in Graphpad Prism 7. Alpha-levels for determination of significance and trends were 0.05 and 0.10, respectively.

4.4. Results

Due to the large number of significant sow × offspring treatment interactions observed, we have focused on the effect of sow or offspring treatment, and have only indicatedif an interaction was also observed, if relevant. While significant interactions are depicted in most of the individual results table/figure, all are summarised in Table S4.4.

4.4.1. Impact of faecal microbiota transplantation on lifetime growth performance of offspring

Offspring growth performance [Average Daily Feed Intake (ADFI), Average Daily Gain (ADG), Feed Conversion Efficiency (FCE), residual feed intake (RFI)] and carcass traits are presented in Table 4.1. Offspring from FMTP sows were 4.3 Kg lighter than offspring from CON sows at 70 days of age ($P<0.05$;), and 8 Kg lighter at ~155 days of age ($P<0.001$). Moreover, FMT-treated offspring were 5.5 Kg lighter than control offspring at ~155 days ($P<0.05$). Carcass weight tended to be lighter for offspring from FMTP sows compared to offspring from CON sows ($P=0.07$). No other growth or carcass parameters examined were significantly affected by either sow or offspring treatment. However, CON/FMT1 and FMTP/CON pigs tended to have a lower RFI (calculated between weaning and slaughter), compared to CON/CON and CON/FMT4 offspring ($P=0.08$).

4.4.2. Analysis of faecal extracts used as inocula

No differences were observed in bacterial load between any of the four donor faecal samples, nor in the inoculum pre- and post-freeze ($P>0.05$; Figure S4.2). Relative abundance of bacterial phyla (n=13) and genera (n=54) in the donor faeces, in samples taken during

aliquoting, as well as in the thawed inocula are shown in Figure S4.2. Inocula samples had similar microbial composition to the donor faeces (Figure S4.2), with *Firmicutes* ranging from 29.0 to 37.9% relative abundance, followed by *Spirochaetes* (21.4-37.1%) and *Bacteroidetes* (14.1- 23.5%). At lower relative abundance, *Chlamydiae* (2.9-12.7%) and *Proteobacteria* (2.8-8.9%) were also observed. The most abundant genera present were those categorised as unclassified (21.4-34.8%), *Treponema* (11.3-21.2%), *Sphaerochaeta* (3.5-22.7%), *Chlamydia* (2.9-12.7%), *Alloprevotella* (3.7-9.7%) and *Prevotella* (4.3-5.9%).

4.4.3. Influence of the FMT procedure on the microbiota of gestating sows

No differences in bacterial load in the baseline faeces collected prior to antibiotic administration were observed between sow treatments. However, following antibiotic treatment, faecal bacterial load was 0.5 $\log_{10}$ copies of 16S rRNA gene/ng DNA lower in FMT sows (the only sows to receive antibiotics; $P<0.05$; Figure 4.2B). Thereafter, total bacterial load was restored due to FMT, as it was higher in post-first FMT compared to post-antibiotic faecal samples ($P<0.05$; Figure 4.2B). In addition, total faecal bacterial load increased from 5.92 $\log_{10}$ copies of 16S rRNA gene/ng DNA pre-antibiotic to 6.27 post-first FMT ($P<0.05$; Figure 4.2B). The antibiotic treatment also reduced faecal microbial diversity, as all three α-diversity indices measured were reduced in FMT sows compared to CON sows ($P<0.05$; Figure 4.2C). Faecal microbial diversity then returned to pre-antibiotic values post-FMT.

In the baseline faeces collected pre-antibiotic, no bacterial phyla differed in relative abundance (Figure 4.2D), but four genera differed in relative abundance between CON and FMT sows: *Butyricimonas*, *Fusobacterium*, *Roseburia*, and *Schwartzia* ($P<0.05$; Table S4.5). Antibiotic administration had the greatest influence on faecal microbial composition, with six

phyla differing in relative abundance in CON versus FMTP sows (*Proteobacteria*, *Lentisphaerae* and *Fibrobacteres* were lower; *Verrucomicrobia*, *Tenericutes* and *Candidatus Saccharibacteria* were higher; Figure 4.2D) and 22 genera affected (13 lower in relative abundance and 9 higher) ($P<0.05$; Table S4.5). Thereafter, FMT appeared to restore the bacterial phylum profile to one similar to the baseline faeces (Figure 4.2D). A higher relative abundance of *Candidatus Saccharibacteria* and *Tenericutes*, and three genera (*Alistipes*, *Citrobacter*, *Ruminococcus*2) was observed after the second FMT in FMTP compared to CON sows ($P<0.05$; Figure 4.2D and E). However, sow faecal diversity was not affected following FMT, nor was microbial diversity of the colostrum ($P>0.05$; Figure 4.2C). A total of 77 OTUs were identified in the colostrum samples, mainly from *Firmicutes* (ranging from 44 to 76% relative abundance), *Proteobacteria* (6-31%), *Bacteroidetes* (3-29%), and *Actinobacteria* (0.01-6.95%) (data not shown). No differences were detected at the phylum level (Figure S4.3), but *Oribacterium* and *Anaerovibrio* were higher in relative abundance in FMTP compared to CON sows due to FMT ($P<0.05$; Figure 4.2E and Figure S4.3).

4.4.4. Faecal Microbiota Transplant-associated impact on microbial diversity in offspring

Bacterial α-diversity, as measured by the Shannon index, was higher in the faeces of 50 day-old offspring from FMTP sows compared to those from CON sows (4.00 vs. 3.47), but was lower in the ileum (1.75 vs. 2.08) (Figure 4.3 and Table S4.4; $P<0.05$). In the ileum, an offspring effect was observed, whereby FMT1 (2.24) offspring had a higher Shannon diversity compared to CON (1.76) or FMT4 (1.77) offspring ($P<0.05$).

Beta-diversity was also investigated throughout the lifetime of the offspring, as illustrated by the PCA plots (Figure S4.4). No differences were detected in the faeces

collected at weaning, or at day 65 or 100 of age, but at day 50, FMT4 offspring clustered away from CON offspring ($P<0.05$). No differences were detected in the ileum, but in the caecum, offspring from FMTP-treated sows clustered away from offspring born to CON sows ($P<0.05$).

4.4.5. Effect of FMT in sows and/or offspring on faecal/intestinal bacterial load and composition in offspring

The total bacterial load in all offspring samples is shown in Figure S4.5. The bacterial load in 65-day old offspring was higher in pigs born to FMTP sows compared to those born to CON sows (6.48 vs. 6.09 $\log_{10}$ copies/ng DNA, $P<0.05$; Figure S4.5), while in the ileum, FMT-treated offspring had a reduced bacterial load compared to CON offspring (3.98 vs 4.44) ($P<0.05$; Figure S4.5).

Significant differences in microbial composition of the faeces and intestinal digesta at both the phylum and genus levels are shown in Figure 4.4. Five bacterial phyla and 16 genera were altered due to a sow × offspring treatment interaction (Table S4.4). Nine of the 14 phyla detected were impacted by the FMTP in sows and eight by FMT in offspring and some of these i.e. *Bacteroidetes* and *Spirochaetes*, were present at high relative abundance.

At weaning, offspring from FMT sows had a higher relative abundance of *Tenericutes* but lower abundance of *Spirochaetes* (Figure 4.4A). *Planctomycetes* was lower but *Lentisphaerae* was higher in abundance in FMT1 offspring compared to the other offspring treatments (Figure 4.4B and Table S4.4). At 50 days of age, offspring from FMTP sows had a lower abundance of *Spirochaetes* but higher abundances of *Proteobacteria* and *Actinobacteria* (Figure 4.4A). Furthermore, *Actinobacteria* were higher in abundance in FMT4-treated offspring compared to CON and FMT1 offspring (Figure 4.4B). At 100 days

of age, the relative abundance of *Synergistetes* was higher while *Lentisphaerae* was lower in offspring from FMTP sows (Figure 4.4A).

In the ileum, *Tenericutes, Chlamydiae* and *Actinobacteria* were less abundant in offspring from FMTP-treated sows (Figure 4.4A and Table S4.4). On the other hand, *Bacteroidetes* were higher in abundance in FMT4 compared to CON and FMT1 offspring, while *Chlamydia* and *Proteobacteria* were higher in FMT1 offspring compared to the other groups (Figure 4.4B). In the caecum, *Verrucomicrobia* was enriched due to FMTP in sows (Figure 4.4A). The effect of offspring treatment was more subtle; *Actinobacteria* was lower, whereas both *Fusobacteria* and *Elusimicrobia* were higher in relative abundance in FMT1 compared to CON and FMT4 offspring (Figure 4.4B). In the colon of offspring, FMTP in sows resulted in a slightly higher abundance of *Fusobacteria* (Figure 4.4A).

Of the 148 genera detected, 16 differed due to sow treatment and 16 due to offspring treatment [mainly at relative abundance <1%; Figure 4.4C and D). At weaning, *Sphaerochaeta* and *Treponema* (from *Spirochaetes*), and *Oribacterium*, *Faecalibacterium* and *Eubacterium* (from *Firmicutes*/*Clostridia*) were less abundant, whereas *Asteroleplasma* was enriched in offspring from FMTP-treated sows (Figure 4.4C and Table S4.4). *Sphaerochaeta* at weaning was also negatively correlated with offspring body weight at slaughter (Figure S4.6). *Streptococcus* was higher in relative abundance in FMT4 offspring compared to CON offspring (Figure 4.4D).

At day 50 of age, *Sphaerochaeta* was less abundant whereas *Campylobacter*, *Alistipes* and *Ruminococcus* were enriched due to FMTP in sows (Figure 4.4C and Table S4.3), although the relative abundance of *Ruminococcus* was reduced in FMT-treated offspring at 65 days of age (Figure 4.4D and Table S4.4). *Bacteroides*, *Anaerostipes* and *Peptococcus* were higher in relative abundance in FMT4 compared to CON pigs (Figure

4.4D). Throughout the growing period similar changes occurred; at day 65, FMT4 offspring had a higher relative abundance of *Peptococcus* compared to CON offspring (Figure 4.4D). *Campylobacter* was higher at day 65 in offspring from FMTP-treated sows (Figure 4.4C), and was at higher abundance at day 100 in FMT4 offspring compared to their control counterparts (Figure 4.4D and Table S4.4). *Peptococcus* was lower in FMT-treated offspring at day 100 of age (Figure 4.4D and Table S4.4). At 65 days of age, *Alloprevotella* was higher in abundance due to FMTP in sows (Figure 4.4C), whereas *Treponema* and *Ruminococcus* were lower in abundance due to FMT4 in offspring (Figure 4.4D). Furthermore, *Terrisporobacter* was lower due to FMT treatment either in sows or offspring (Figure 4.4C and D). In both the faeces collected at day 100 and the caecal digesta, *Fusobacterium* was less abundant in FMT4 compared to FMT1 offspring (Figure 4.4D).

Most of the genus-level differences between treatments occurred in the ileum of offspring (n=10; Figure 4.4C, D and Table S4.4). *Prevotella* and *Oscillibacter* were higher in relative abundance, whereas *Asteroleplasma*, *Blautia*, *Butyricimonas* and *Veillonella*, were lower due to FMTP in sows (Figure 4.4C). Ileal *Prevotella* was also negatively correlated with offspring final body weight also (Figure S4.6). *Sphaerochaeta* was lower in abundance in FMT4 compared to CON offspring, while *Blautia*, *Butyricimonas*, *Butyricicoccus*, *Faecalibacterium*, *Gemmiger*, and *Oribacterium* were higher in abundance due to FMT1 in offspring (Figure 4.4D and Table S4.4). No treatment differences were observed for genera in the colon.

4.4.6. Influence of FMT in sows and/or offspring on *in silico* predictions of bacterial functionality in offspring faecal/intestinal samples

The impact of FMT on predicted functionality of offspring faecal and intestinal microbiota is shown in Figure 4.5. Due to a sow x offspring treatment interaction, 35 predicted pathways were affected (Table S4.4), with with 8 impacted due to FMTP in sows (Fig 4.5A) and 17 due to FMT in offspring (Fig. 4.5B). These belonged to 11 general categories, mainly carbohydrate (n=4), amino acid and lipid metabolism (n=3, respectively) and were mostly in the ileum (n=20).

In faeces at 50 days of age, offspring from FMTP sows had a lower predicted abundance of phosphonate and phosphinate metabolism pathways, compared to offspring from CON sows, with the opposite occurring in the ileum (Figure 4.5A), although abundance was also lower due to FMT4 in offspring (Figure 4.5B). However, N-glycan biosynthesis pathways were more abundant in the faeces of the offspring from FMTP sows at 50 and 65 days of age and in the colon (Figure 4.5A) but were lower in the ileum of FMT1 offspring (Figure 4.5B). All of the other differences in predicted microbial function observed at sow and offspring treatment level occurred in the ileum. Here, pathways relating to lipid metabolism i.e. alpha-linolenic acid metabolism, primary and secondary bile acid biosynthesis and steroid biosynthesis, were less abundant in offspring from FMTP versus CON sows (Figure 4.5A and Table S4.4). However, the latter two were predicted to be more abundant in FMT1 offspring compared to the other offspring treatment groups (Figure 4.5B and Table S4.4). The naphthalene degradation pathway was enriched in offspring from FMTP sows (Figure 4.5A and Table S4.4), and similarly, the styrene degradation pathway was higher in abundance in FMT-treated offspring (Figure 4.5B and Table S4.4). While one pathway relating to carbohydrate metabolism (pentose and glucuronate interconversions) was more abundant in offspring due to FMTP in sows (Figure 4.5A and Table S4.4), others

(propanoate metabolism, pentose phosphate, pyruvate metabolism) were less abundant in FMT4 offspring. Similarly, pathways relating to amino acid metabolism (tryptophan metabolism, amino acid-related enzymes and phenylalanine, tyrosine and tryptophan biosynthesis), tetracycline biosynthesis, methane and nitrogen metabolism, protein kinases, and pantothenate and CoA biosynthesis were all predicted to be less abundant in FMT4 offspring (Figure 4.5B and Table S4.4). Notably, the pathway predicted at the highest relative abundance (stilbenoid, diarylheptanoid and gingerol biosynthesis) was more abundant in FMT-treated offspring than in the other offspring treatments (Figure 4.5B).

4.4.7. Effect of FMT in sows/and/or offspring on intestinal volatile fatty acid concentrations

Volatile fatty acid (VFA) concentrations were measured in the ileal, caecal and colon digesta (Figure S4.7 and Table S4.4). In the ileum, propionic acid was ~1.4 fold higher in offspring from FMT-treated sows, and butyric acid concentrations were ~2-fold higher in FMT-treated offspring compared to control pigs ($P<0.05$). No differences were found in the caecum, but FMT in sows caused a 1.8-fold reduction in the concentration of isobutyric acid in the colon digesta of offspring ($P<0.05$).

4.4.8. Effect of FMT in sows/and/or offspring on intestinal histology in offspring

Histological examination of tissue from the duodenum, jejunum and ileum revealed FMT-associated differences in the offspring (Figure 4.6). The number of duodenal goblet cells was lower ($P<0.05$; Figure 4.6F), and the jejunal villus height to crypt depth ratio was reduced ($P<0.05$; Figure 4.6D) due to FMT in sows. Furthermore, ileal villus height, width and area were reduced ($P<0.05$; Figure 4.6A and E) and a higher number of ileal goblet cells per µm

villus height was observed in offspring from FMT sows (P<0.05; Figure 4.6G). Despite a lack of FMT-associated differences in the villus height to crypt depth ratio, ileal crypt depth was shorter due to FMT in sows (P<0.05; Figure 4.6B, C, and E).

4.4.9. Effect of FMT in sows/and/or offspring on duodenal enzyme activity and gene expression, and blood parameters

Disaccharidase enzyme activity and expression of selected genes, both measured in the duodenal mucosa of offspring are shown in Figure 4.7. Of the three brush border enzymes measured, only maltase activity was affected, tending to be 1.5-fold lower in offspring due to FMT in sows (P=0.08; Figure 4.7A).

Regarding gene expression, FMT in sows resulted in up-regulation of the genes encoding the tight junction protein zona occludens 1 (*ZO1*) as well as toll-like receptor 2 (*TLR2*) in offspring compared to offspring from CON sows (P<0.05; Figure 4.7B). Likewise, the tight junction protein, occludin encoding gene (*OCLN*) was up-regulated in offspring due to FMT in sows or offspring (P<0.05).

No interaction effect was observed for any of the serum biochemistry parameters measured at slaughter (P>0.05; Table S4.6), but FMT in sows led to lower cholesterol concentrations in offspring (P<0.05). On the other hand, FMT-treated offspring had lower serum total protein concentrations compared to control offspring (P<0.05).

Haematological parameters measured at slaughter are shown in Table S4.7. The only treatment effect observed was that offspring from FMT-treated sows had lower mean corpuscular volume and mean platelet volume than offspring from CON sows (P<0.05).

4.5. Discussion

As the work from Chapters 2 and 3 have identified a link between the porcine intestinal microbiota and FE, this work investigated, for the first time, the use of FMT as a tool to manipulate the intestinal microbiota in order to improve FE in pigs. However, contrary to our hypothesis, transplantation of microbiota from highly feed efficient pigs (Chapter 3) into gestating sows and/or their offspring resulted in a depression in offspring weight. This became apparent 42 days after the last inoculation and persisted through to slaughter at ~155 days of age. Conversely, in terms of FE, RFI tended to be lower (FE improved) in control offspring from FMT sows, and in offspring receiving the FMT just once, born to control sows.

Despite the negative impact on growth, FMTP appeared to be an effective means of repopulating the sow intestine following antibiotic treatment, allowing recovery of both the bacterial load and diversity. Compositional differences were also evident within the sow faecal microbiota post-FMT, but to a lesser extent than those seen after antibiotic treatment, and microbial diversity was not impacted. Nonetheless, although the faecal microbiota composition of the FMT-treated sows appeared to revert to baseline at the phylum level, there were three genera found which did not differ at baseline; *Alistipes*, *Ruminococcus*2 and *Citrobacter* were enriched as a result of FMT. *Alistipes* has previously been suggested as FE-enhancing in chickens (Torok et al., 2011), which may help to explain the FMT-associated improvements in RFI observed in the present study, and *Ruminococcus*2 plays a central role in the fermentation of complex carbohydrates (Salonen et al., 2014). *Alistipes* and *Ruminococcus* (albeit not *Ruminococcus*2) were also enriched in the offspring from FMT-treated sows. This, together with the increased bacterial load and faecal diversity found in these offspring (albeit only at certain time points), suggests a possible beneficial maternal influence, as increased intestinal microbial diversity is associated with improved gut health in

humans (Le Chatelier et al., 2013) and reduced susceptibility to post-weaning diarrhoea in pigs (Dou et al., 2017). On the other hand., *Citrobacter* comprises species that are opportunistic pathogens in humans (Ranjan et al., 2013), and although no link has been made in pigs to date (Schierack et al., 2007) this may suggest transfer of an "undesirable" microbiome to offspring, which could account for the depression in offspring body weight.

Colostrum likely acts as a vehicle for microbiota transfer to offspring (Fernandez et al., 2013, Maradiaga et al., 2014). Here, it appears that FMT impacted the bacterial profile of colostrum, albeit only to a limited extent, as only two relatively low abundance genera were affected. Furthermore, these FMT-associated compositional differences in the colostrum do not seem to have been carried through to the offspring. *Oribacterium,* for example, was more abundant in the colostrum from FMT-treated sows but less abundant in the faeces of pigs weaned from these sows (but more abundant in the ileum of finisher pigs treated once with FMT).

The FMT had its greatest impact in the ileum of the offspring, influencing microbiota composition, predicted functionality, VFA concentrations and histology. Biologically, this is of major relevance, given the importance of the ileum in terms of nutrient absorption and immune response (Wijtten et al., 2011). A higher abundance of *Bacteroidetes* was observed in the ileum of FMT4 offspring, and increased faecal *Bacteroidetes* has previously been linked with lower fatness in pigs (Pedersen et al., 2013, Yang et al., 2016). Additionally, the increased abundance of *Bacteroides* in the 50-day old offspring, and of *Prevotella* in the ileum, mediated by both FMT in sows and offspring, is in accordance with lower fatness in pigs, as previously documented (Yang et al., 2016) and may also help to explain the reduction in offspring body weight found.

Butyric acid producers, linked with improved gut health and metabolism, including *Faecalibacterium*, *Oribacterium* and *Eubacterium* from *Clostridiales* (Lee et al., 2017, Niu et al., 2015, Heinritz et al., 2016), were impacted due to FMT. For example, the reduction in *Faecalibacterium*, known for its anti-inflammatory properties, and linked with heavier body weight in pigs (Heinritz et al., 2016, Han et al., 2016), may help to explain the FMT-associated reduction in body weight observed. Furthermore, multiple inoculations in offspring may have amplified the negative impact as the abundance of butyrate producers (i.e. *Butyricicoccus, Butyricimonas, Faecalibacterium*, *Gemmiger*, *Oribacterium*) was reduced in the ileum of offspring that received FMT four times compared to offspring receiving FMT only once.

It is notable that a number of health-related predicted microbial pathways such as those associated with lipid metabolism (α-linolenic acid, primary and secondary bile and steroid biosynthesis) and biosynthesis of other secondary metabolites (stilbenoid, diarylheptanoid and gingerol biosynthesis), were reduced in the ileum of offspring due to FMT. Some of these pathways may have anti-inflammatory effects (Yadav et al., 2003, Calder, 2001, Zhu et al., 2016) and could be associated with pig growth, as the stilbenoid, diarylheptanoid and gingerol biosynthesis pathway was enriched in high body weight rabbits (Zeng et al., 2015). In contrast, pathways related to xenobiotics biodegradation, and N-glycan biosynthesis were increased in abundance due to FMT, and the latter may be related to the higher abundance of *Campylobacter* found in these pigs. A similar link was previously made in rabbits (Zeng et al., 2015), as the N-glycan biosynthesis pathway was first identified in *Campylobacter jejuni* (Szymanski et al., 1999).

It is difficult to attribute the FMT-associated differences in VFA production to a particular phylotype. However, our microbiota data suggest a rise in propionic acid

producers (mostly members of *Bacteroidetes* and some from *Firmicutes*) in the ileum due to FMT, accompanied by higher ileal propionic acid concentrations. Similarly, several butyric acid producers (*Butyricicoccus*, *Gemmiger*, *Oscillibacter*, and *Faecalibacterium*) were enriched due to FMT, along with a concomitant increase in ileal butyric acid concentrations in FMT-treated offspring. Butyric acid is generally associated with improved gut health (Rivera-Chavez et al., 2016, Kien et al., 2007). However, due to its role in regulating cytokine production (Cushing et al., 2015), one could speculate FMT-associated activation of the immune system, and so diversion of energy away from weight gain. Furthermore, the increased duodenal expression of *TLR2* in offspring from FMTP sows, suggests increased innate host defence signalling, possibly triggered by the altered intestinal microbiota (Aliprantis et al., 1999, Schwandner et al., 1999).

Although limited information exists relating the microbiota with intestinal structure and function, increased abundance of members of *Clostridia* have been correlated with increased ileal villus height in pigs (Suo et al., 2012). This is in agreement with our findings; we found a lower abundance of genera within *Clostridia* and reduced villus height. Interestingly, an FMT-associated up-regulation of *OCLN* and *ZO1* genes, encoding tight junction proteins involved in regulating mucosal barrier function (Chen et al., 2013, Ballard et al., 1995) was observed, suggesting a more selective duodenal paracellular permeability, possibly leading to impaired absorptive capacity. Additionally, the greater number of goblet cells may indicate over-production of mucin in the ileum and so an over-enhanced barrier function, a possible response to "foreign" microbes from the faecal inoculum. Increased mucin secretion may also result in decreased nutrient absorption, as excess mucin acts as a physical barrier (Montagne et al., 2004). Moreover, work from Chapter 2 showed that high RFI (less feed efficient) pigs had more ileal goblet cells. Similarly, the FMT-associated

reductions in ileal villus height and area are likely to have decreased nutrient absorption (Pluske et al., 1997). Taken together, these findings may help to explain the FMT associated reduction in offspring body weight observed. Additionally, FMT was associated with reduced serum protein and cholesterol concentrations, which may be a consequence of decreased intestinal absorption. Interestingly, FMT in sows resulted in higher ileal propionic acid concentrations in their offspring, and propionate has the potential to lower blood cholesterol, as found in rats (Wright et al., 1990).

The negative impact of FMT observed in the present study could be attributed to the inoculum used. It comprised *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, the predominant bacterial phyla within the faecal microbiome of pigs (Zhao et al., 2015, Kim and Isaacson, 2015a). However, *Spirochaetes* and *Chlamydiae*, members of which are pathogenic to pigs (Englund et al., 2012), were both higher in relative abundance in the donor faeces than usually found in finisher pigs (Kim et al., 2011), and this was reflected in the inoculum and faeces of sows and offspring receiving the FMT. This raises a question regarding donor suitability, as the donors were finisher pigs (~130 days of age) and recipients were adult sows and neonatal piglets (the latter with a naïve/essentially non-existent microbiome. The issue of 'non-ideal' donors has been highlighted recently in humans, whereby a woman developed new-onset obesity after FMT from a healthy, but overweight donor (Alang and Kelly, 2015). Furthermore, there is always a concern that pathogens can be transferred to the recipients (Pamer, 2014) and the donor pigs used in the present study, although healthy and highly feed efficient, were not screened for pathogens.

4.6. Conclusion

In conclusion, FMT in sows during late gestation and in neonatal offspring had a detrimental effect on offspring lifetime growth. In general, bacteria (and their predicted

pathways) with a role in nutrient utilization and intestinal health, were less abundant as a result of FMT. This may relate to altered physiological parameters, as intestinal morphology was negatively impacted, and intestinal permeability- and VFA transport-related mucosal gene expression was altered. Together, these data suggest reduced nutrient absorptive capacity and a less metabolically efficient microbiome, possibly explaining the reduced weight at slaughter. Overall, although the findings of this study demonstrate the impact of early life intestinal microbiota on pig growth, FMT proved ineffective as a strategy to improve FE in pigs, possibly due to adverse effects on microbiota composition resulting from the use of non-age-matched donors. Targeted nutritional strategies aimed at increasing/decreasing specific FE-associated taxa may be more appropriate, perhaps using bacterial taxa present in the control offspring from the present study.

4.7. Tables and Figures

Table 4.1. Effect of faecal microbiota transplantation (FMT) in sows and/or offspring on growth performance and carcass traits in offspring[1]

Sow treatment	Control[2]			FMT[3]			S.E.M	P-value		
Offspring treatment	Control[4]	FMT1[5]	FMT4[6]	Control	FMT1	FMT4		Interaction	Sow	Offspring
Weight (kg)										
Birth	1.38	1.34	1.36	1.32	1.34	1.33	0.059	0.41	0.93	0.71
Weaning	8.8	8.2	8.0	7.6	7.2	7.0	2.64	0.99	0.52	0.93
Day 70	35.2^{a}	29.9^{b}	30.4^{b}	29.1^{b,c}	27.1^{c,d}	26.4^{d}	2.29	0.01	0.02	0.19
Day 155	127.9^{a}	116.0^{c}	120.9^{b}	115.3^{c}	114.2^{c}	111.3^{c}	2.87	<0.001	<0.001	<0.001
Weaning to 155 days of age										
ADFI[7] (g/day)	1974	1789	1924	1790	1850	1851	65.6	0.17	0.25	0.51
ADG[8] (g/day)	926	850	901	873	884	870	29.7	0.31	0.51	0.56
FCE[9] (g/g)	2.08	2.04	2.06	2.02	1.95	2.16	0.639	0.28	0.72	0.22
RFI[10] (g/day)	21.2^{A}	-30.2^{B}	24.6^{A}	-30.1^{B}	9.4^{A,B}	1.33^{A,B}	21.38	0.08	0.51	0.52
Carcass traits										
Carcass weight (kg)	97.0	89.1	92.8	89.1	86.2	90.9	2.85	0.54	0.07	0.14
Kill out yield (%)	76.7	76.5	76.5	76.4	76.2	76.4	0.29	0.88	0.39	0.80
Fat (mm)	16.3	15.7	15.1	14.8	14.9	15.9	0.73	0.29	0.41	0.93
Muscle depth (mm)	52.3	50.5	50.4	52.1	51.8	53.0	1.53	0.64	0.33	0.78
Lean meat yield (%)	54.3	54.4	54.9	55.3	55.2	54.5	0.63	0.54	0.36	0.97

[1]Least squares means and pooled standard error of the mean is presented.

Sows were assigned to one of two treatment groups: [2]Control (n=9) and [3]FMT procedure (FMTP; n=9); FMT sows received FMT via gastric intubation on days 70 and 100 of gestation. Piglets were assigned to one of three treatment groups at birth: [4]Control; [5]FMT1 (FMT at birth) and [6]FMT4 (FMT at birth, days 3, 7 and 28 of age).[7]ADFI: average daily feed intake; [8]ADG: average daily gain; [9]FCE: feed conversion efficiency; [10]RFI: residual feed intake, calculated between day 28 and 155 days of age, using a least squares multiple-regression model of ADFI on ADG, metabolic live weight, sex and all relevant two-way interactions, as well as the effects of back-fat and muscle depth, which were recorded at slaughter.

Days presented in the table correspond to days of age.

Data from 74 pigs: Sow treatment level: control n=39; FMTP n=35; Offspring treatment level: control n=24; FMT1 n=25; FMT4 n=25.

[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P<0.05$).

[A,B,C] Within each row, values that do not share a common superscript tend to be different ($P<0.10$).

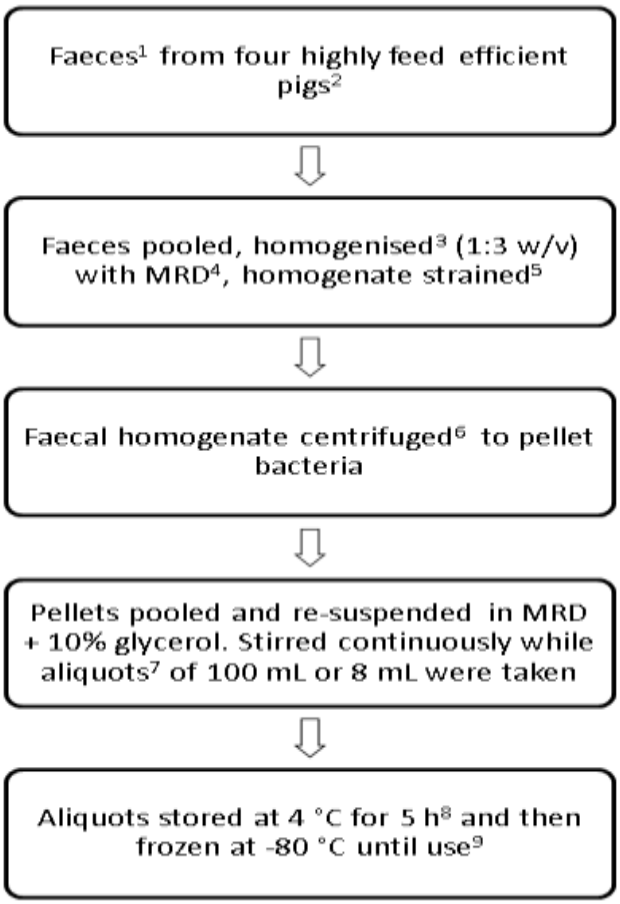
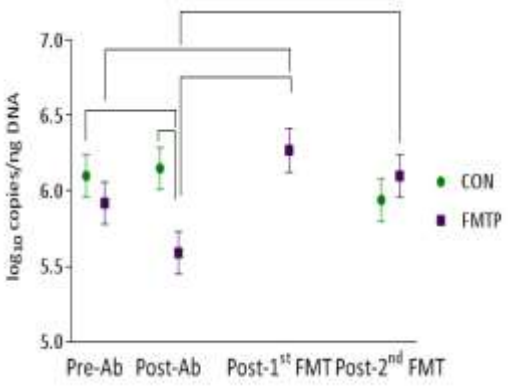


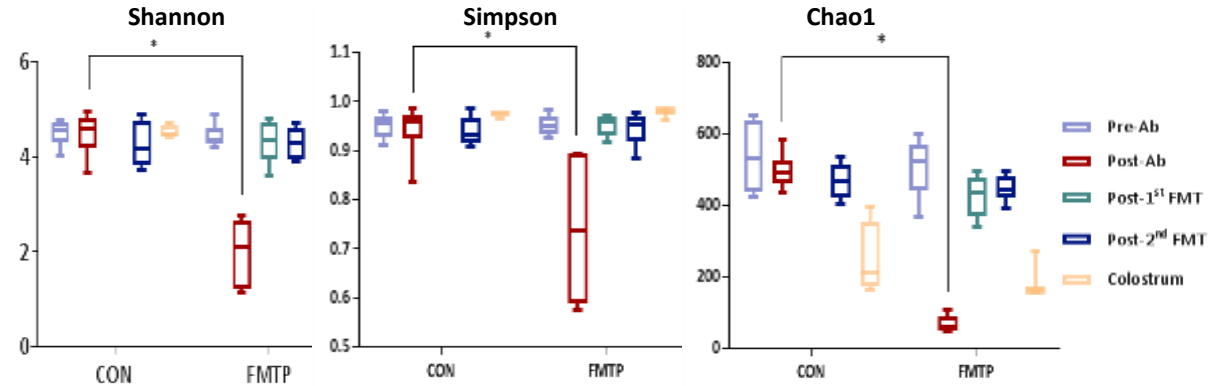
Figure 4.1. Schematic illustration depicting preparation of faecal inoculum used for faecal microbiota transplantation (FMT) based on a method developed by O'Donnell et al., (2016)

[1]Collected by rectal stimulation directly into sterile bags, placed into anaerobic jars and processed immediately in an anaerobic workstation. Sub-samples were collected from each faecal sample, snap frozen and stored at -80 °C for microbiota analysis; [2]Four finisher pigs (130 days of age) with the lowest residual feed intake (i.e. the most feed efficient) from a previous study comprising 409 pigs from Chapter 3; [3]1,000 rpm for 10 min using a magnetic stirrer;[4]Maximum recovery diluent (Oxoid Ltd, Basingstoke, Hampshire); [5]Strained through a sieve to remove large particles, which were then washed through the sieve with another part of MRD; [6]30 min at 4,000 × *g*, supernatant discarded; [7]Aliquots of 100 mL were taken for sow FMT and aliquots of 8 mL (in 10 mL syringes) were taken for offspring FMT. Sub-samples were collected at the start and the end of the aliquoting process, snap frozen and stored at -80 °C for microbiota analysis; [8]To allow microbial adjustment to low temperatures; [9] Stored for no longer than 3 months at -80 °C.

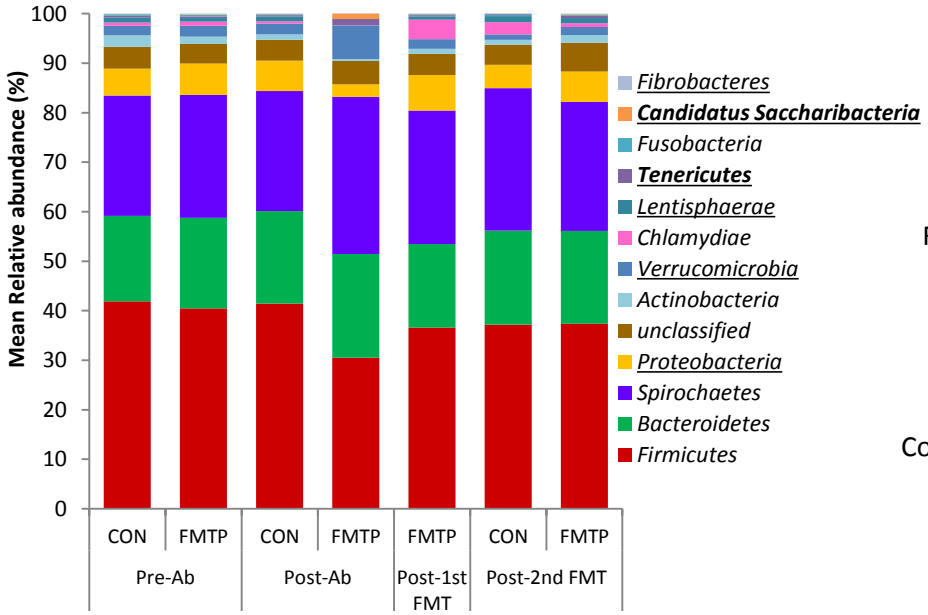
B. Total bacterial load in sow



C. Alpha diversity within sow faeces and colostrum



D. Bacterial phyla in sows across all faecal time points during



E. Compositional differences within sow faecal microbiota post-2nd FMT and within colostrum microbiota

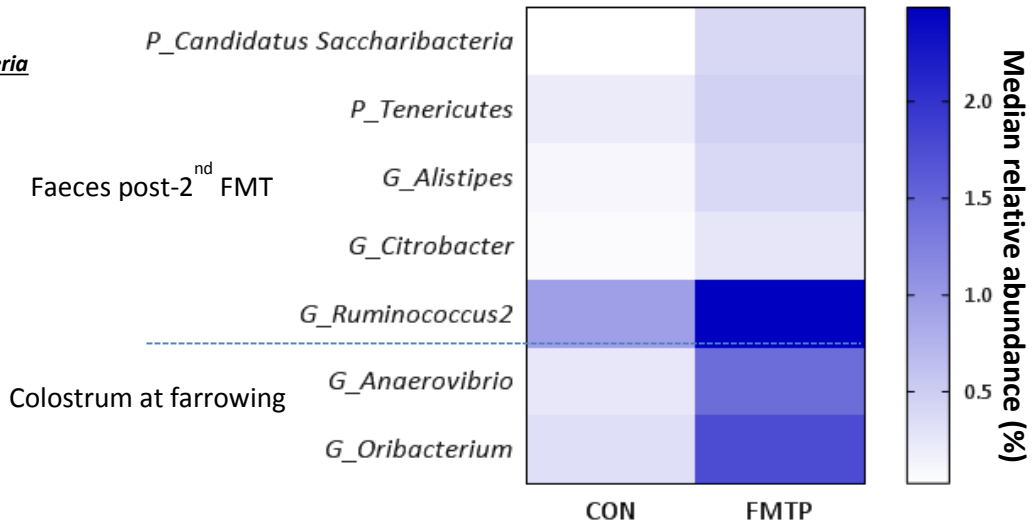


Figure 4.2. A. Sow sampling timeline. Effect of antibiotic administration and faecal microbiota transplantation (FMT) in sows during gestation on B. Total bacterial load in the faeces, C. Faecal and colostrum microbial diversity (at the genus level) and D. Composition of bacterial phyla during gestation. E. Compositional differences within the faecal microbiota post-second FMT and within colostrum microbiota at farrowing (P<0.05) are also shown

Sow faeces: control (CON) n=6; FMT procedure (FMTP) n=6. Sow colostrum: control (CON) n=4; FMT n=3.
Pre-Ab: Pre-antibiotic; Post-Ab: 4 days post-antibiotic; Post-1stFMT: 30 days after 1st FMT at day 100 of gestation; Post-2nd FMT: 12 days after 2nd FMT at day 112 of gestation.
*Indicates significant differences (P<0.05).
Phyla underlined in panel C indicate those that differed in FMTP versus CON sows due to antibiotic treatment, and phyla in bold are those that differed post 2nd-FMT.
[1]Post 1st-FMT faecal sample: only collected from FMTP sows.

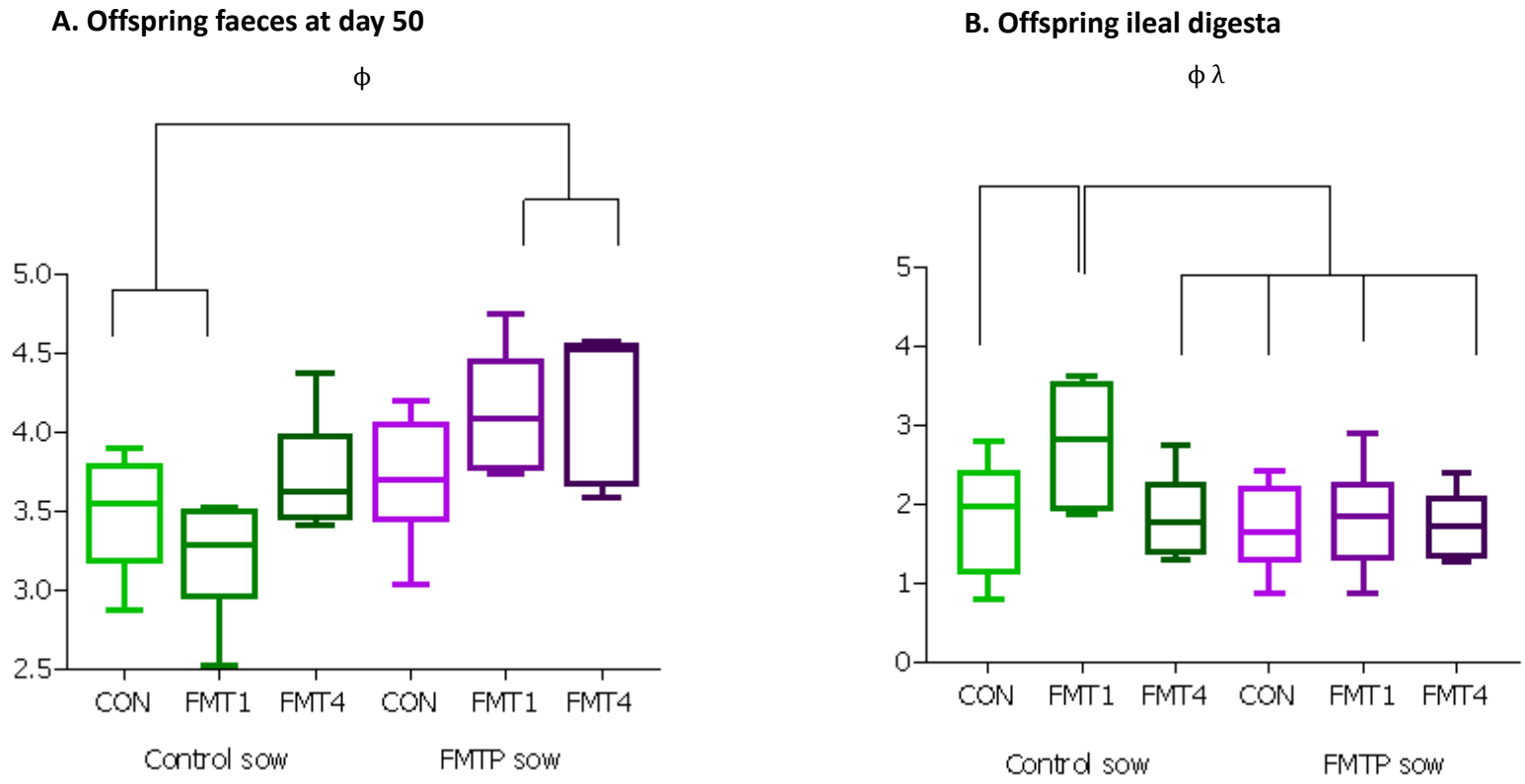
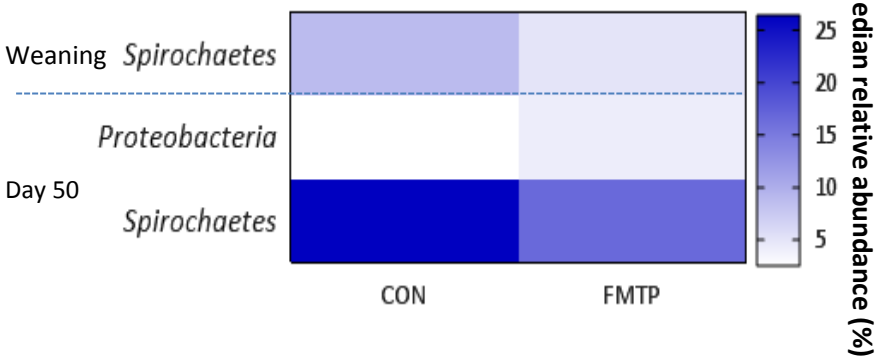


Figure 4.3. Variations in *α*-diversity (at the genus level) of the offspring microbiota in A. Faeces at 50 days of age and B. Ileal digesta at ~155 days of age as a result of faecal microbiota transplantation (FMT) in sows and/or offspring (P<0.05)

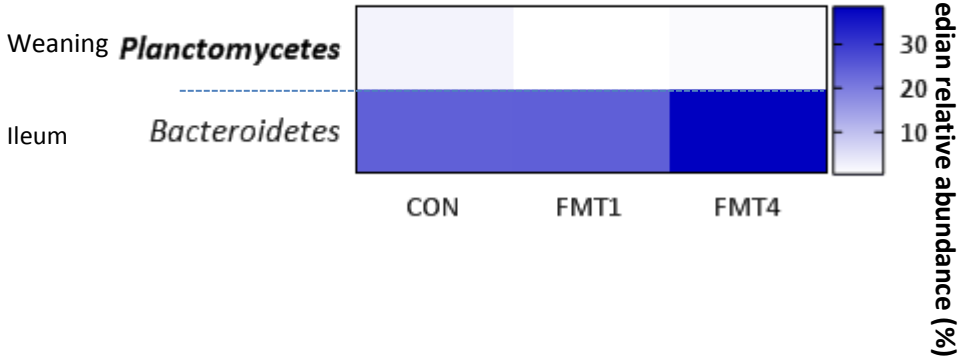
*Indicates significant differences at sow × offspring treatment level (P≤0.05); φ indicates sow treatment effect (P≤0.05); λ indicates offspring treatment effect (P≤0.05).

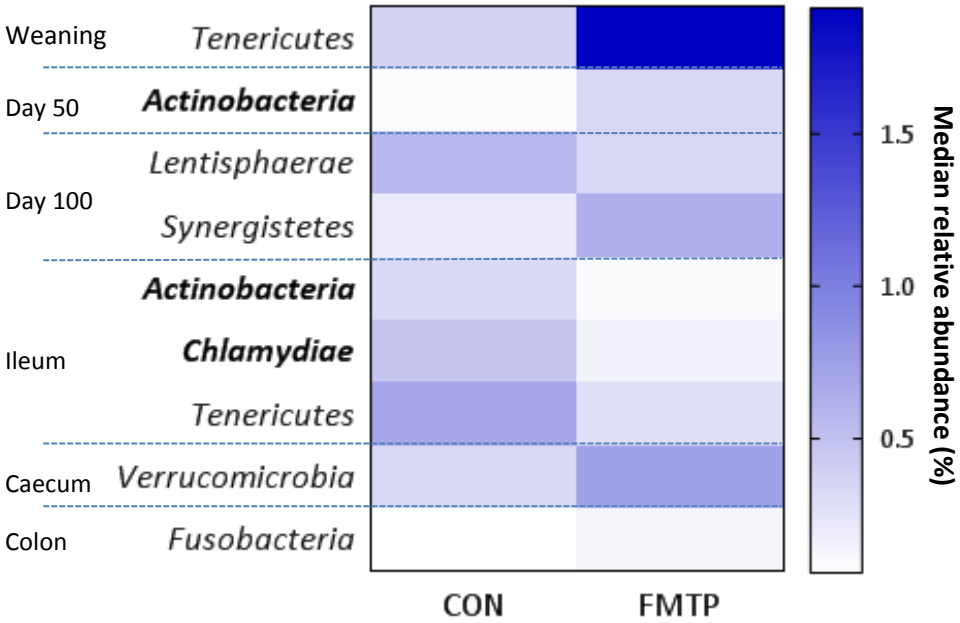
Data from 36 pigs: Sow treatment level control (CON) n=18; FMT n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12.

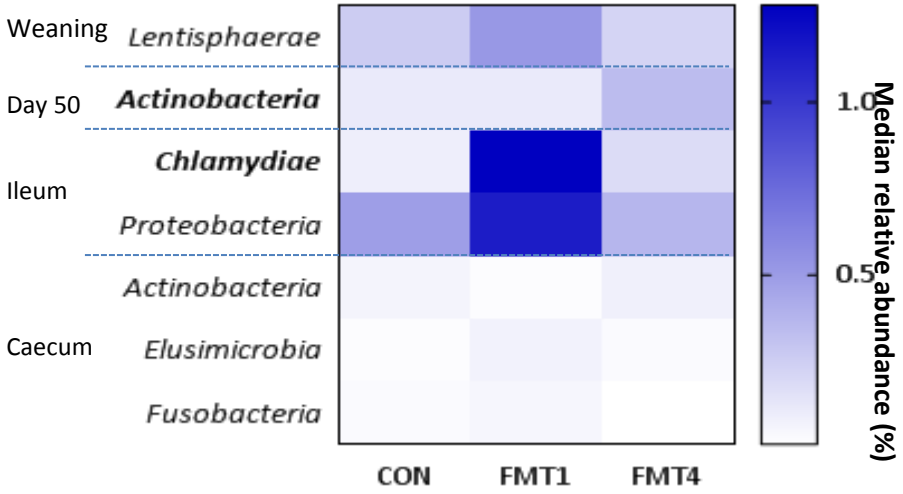
A. Phyla at sow treatment level



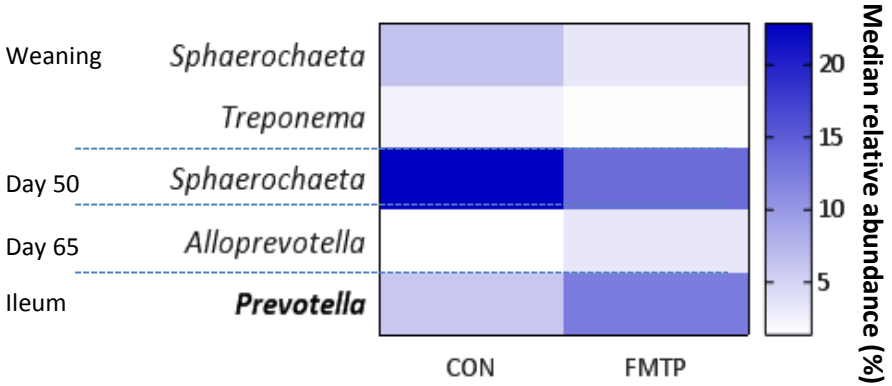
B. Phyla at offspring treatment level



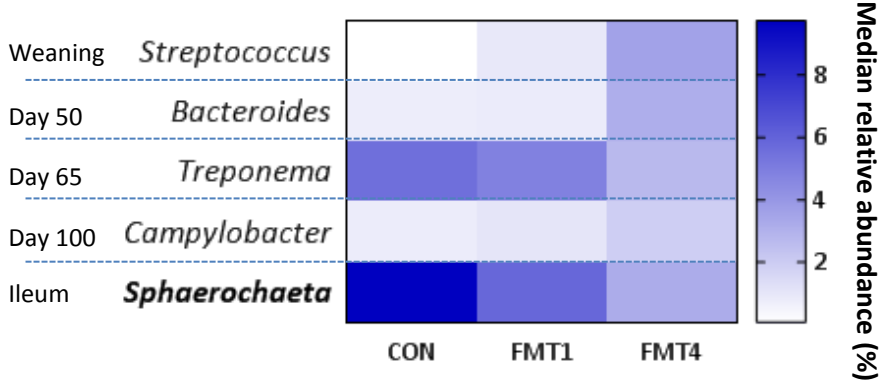


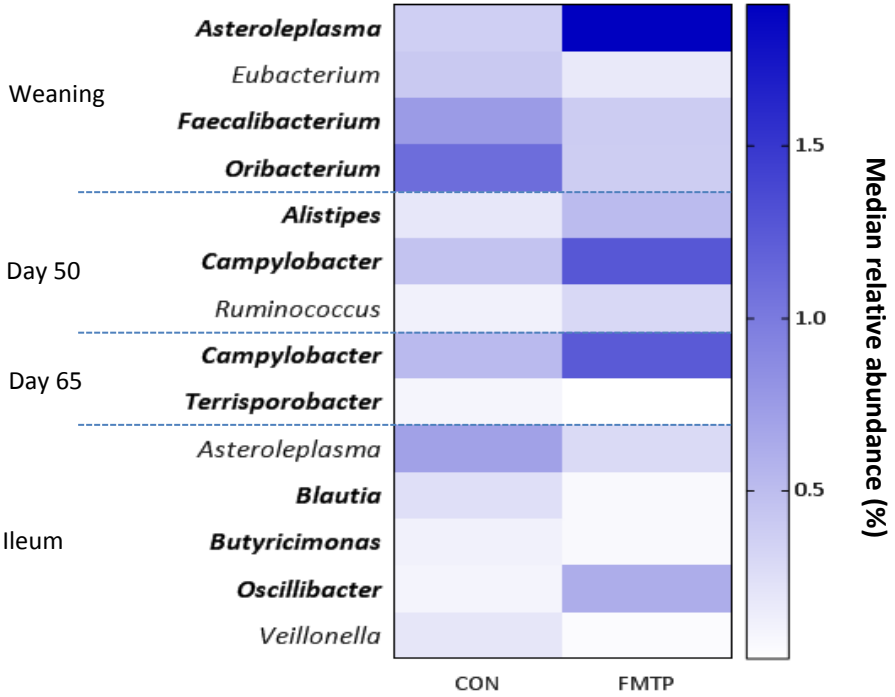


C. Genera at sow treatment level



D. Genera at offspring treatment level





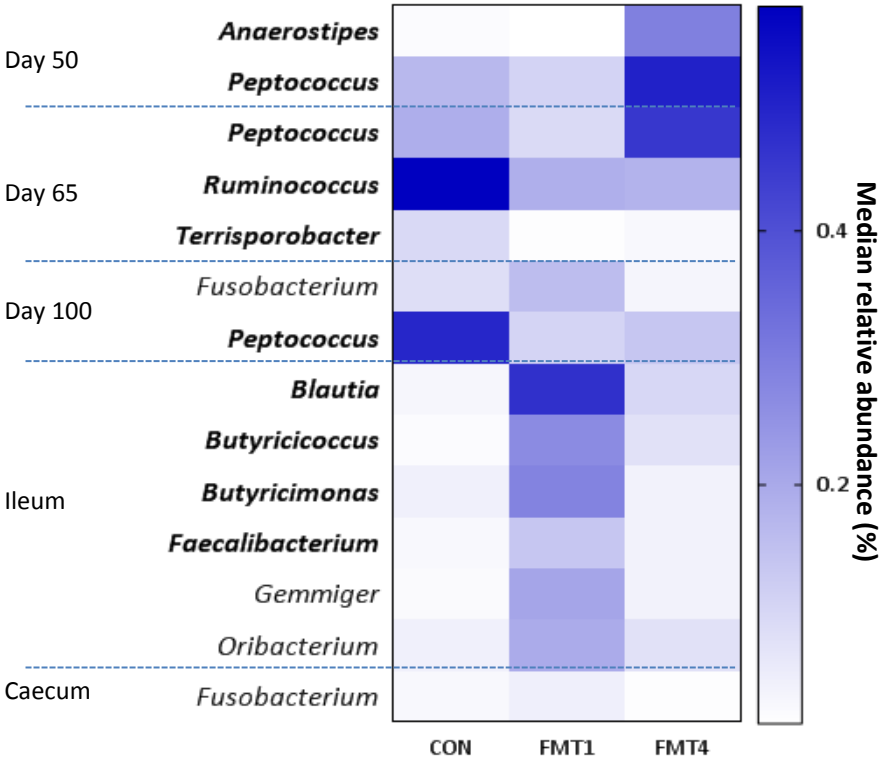


Figure 4.4. Effect of faecal microbiota transplantation (FMT) in sows and/or offspring on median relative abundance (%) of bacterial phyla in faeces and digesta at A. sow treatment level and B. offspring treatment level and of bacterial genera at C. sow treatment level and D. offspring treatment level

Data from 36 pigs: Sow treatment level control (CON) n=18; FMT procedure (FMTP) n=18; Offspring treatment level control (CON) n=12; FMT1 n=12; FMT4 n=12.

Heatmaps are split by relative abundance, with higher abundance phyla/genera shown in the upper heatmaps, and lower abundance taxa shown in lower heatmaps.

Phyla and genera that are in bold depict those affected by a sow treatment × offspring treatment interaction. These, together with other phyla and genera also affected by a sow treatment × offspring treatment interaction are shown in Table S4.3.

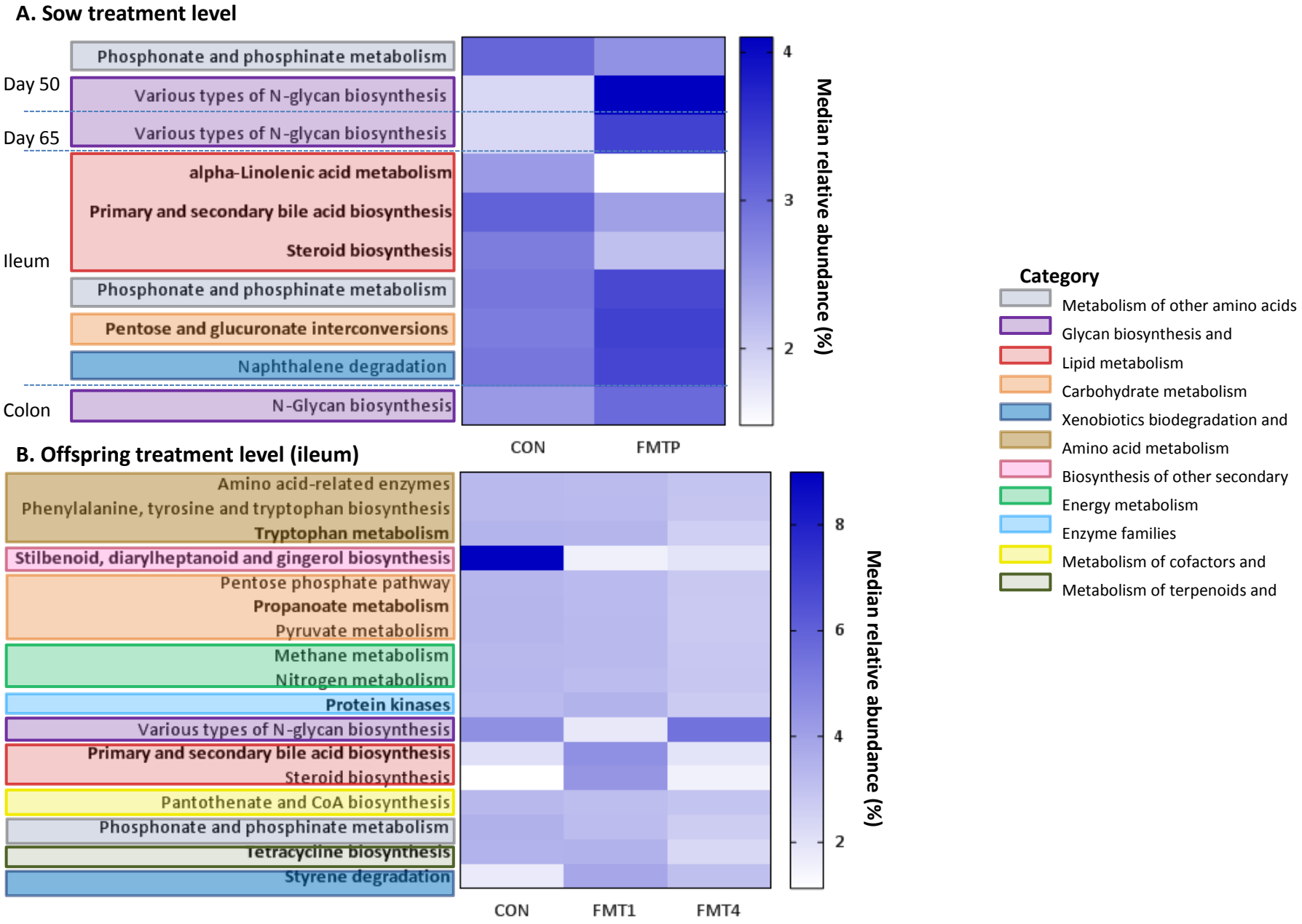

A. Sow treatment level
Phosphonate and phosphinate metabolism
Day 50
Various types of N-glycan biosynthesis
Day 65
Various types of N-glycan biosynthesis
alpha-Linolenic acid metabolism
Primary and secondary bile acid biosynthesis
Steroid biosynthesis
Ileum
Phosphonate and phosphinate metabolism
Pentose and glucuronate interconversions
Naphthalene degradation
Colon
N-Glycan biosynthesis
4
3
2
Median relative abundance (%)
CON
FMTP
B. Offspring treatment level (ileum)
Amino acid-related enzymes
Phenylalanine, tyrosine and tryptophan biosynthesis
Tryptophan metabolism
Stilbenoid, diarylheptanoid and gingerol biosynthesis
Pentose phosphate pathway
Propanoate metabolism
Pyruvate metabolism
Methane metabolism
Nitrogen metabolism
Protein kinases
Various types of N-glycan biosynthesis
Primary and secondary bile acid biosynthesis
Steroid biosynthesis
Pantothenate and CoA biosynthesis
Phosphonate and phosphinate metabolism
Tetracycline biosynthesis
Styrene degradation
8
6
4
2
Median relative abundance (%)
CON
FMT1
FMT4
Category
Metabolism of other amino acids
Glycan biosynthesis and
Lipid metabolism
Carbohydrate metabolism
Xenobiotics biodegradation and
Amino acid metabolism
Biosynthesis of other secondary
Energy metabolism
Enzyme families
Metabolism of cofactors and
Metabolism of terpenoids and

Figure 4.5. Effect of faecal microbiota transplantation (FMT) in sows and/or offspring on median relative abundance (%) of predicted functional pathways in offspring faecal and intestinal microbiota at A. sow and B. offspring treatment level

Data from 36 pigs: Sow treatment level control (CON) n=18; FMT procedure (FMTP) n=18; Offspring treatment level control (CON) n=12; FMT1 n=12; FMT4 n=12.

Predicted pathways that are in bold depict those affected by a sow treatment × offspring treatment interaction. These, together with other predicted pathways also affected by a sow treatment × offspring treatment interaction are shown in Table S4.3.

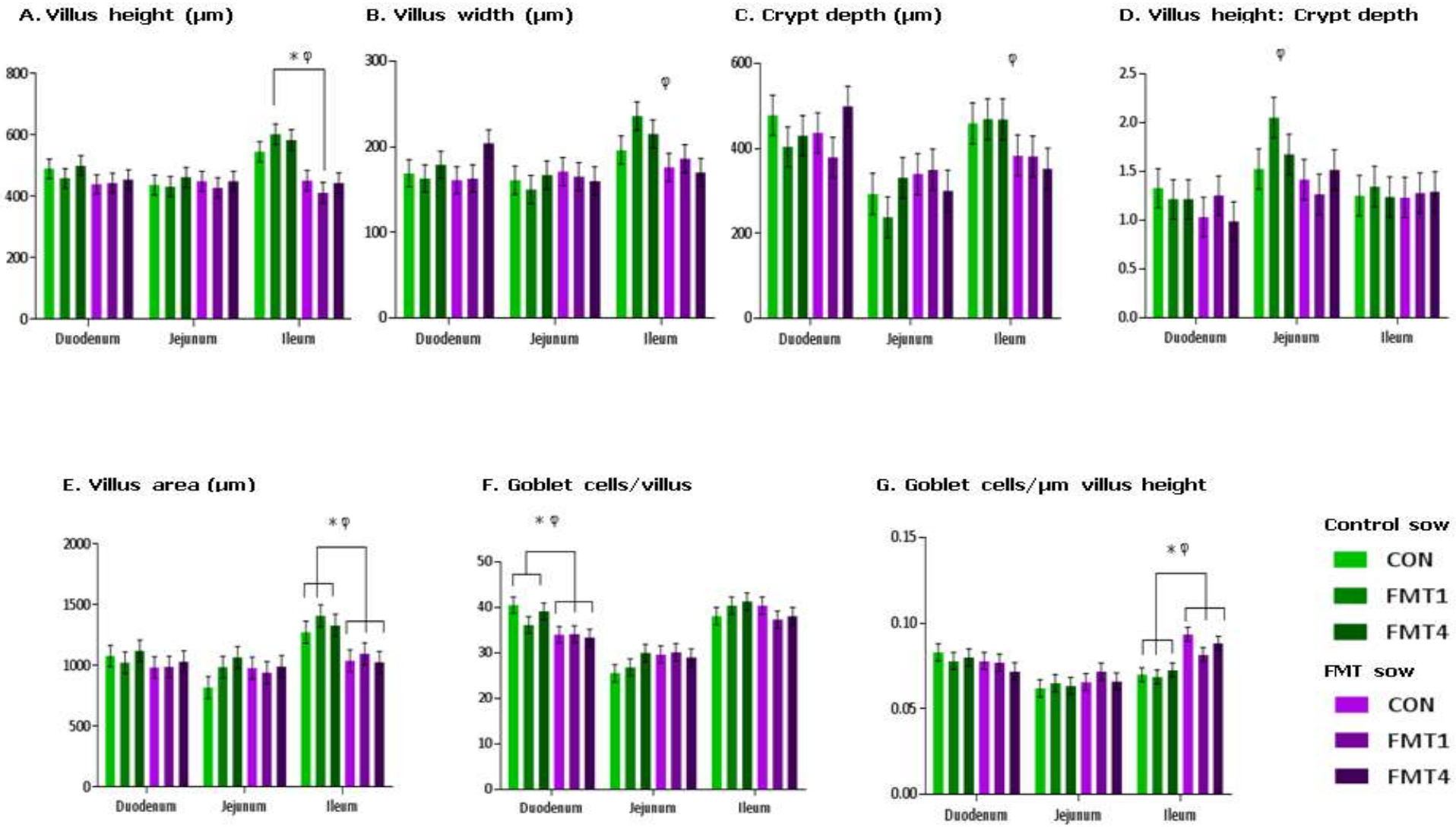

A. Villus height (µm)
B. Villus width (µm)
C. Crypt depth (µm)
D. Villus height: Crypt depth
E. Villus area (µm)
F. Goblet cells/villus
G. Goblet cells/µm villus height
Duodenum
Jejunum
Ileum
Control sow
CON
FMT1
FMT4
FMT sow
CON
FMT1
FMT4

Figure 4.6. Effect of faecal microbiota transplantation (FMT) in sows and/or offspring on offspring intestinal histology; A. Villus height, B. Villus width, C. Crypt depth, D. Villus height: crypt depth ratio, E. Villus area, F. Number of goblet cells per villus, and G. Number of goblet cells per µm villus height

*Indicates significant differences at sow x offspring treatment level ($P \leq 0.05$); φ indicates sow treatment effect ($P \leq 0.05$).

Data from 36 pigs: Sow treatment level control (CON) n=18; FMT procedure (FMTP) n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12.

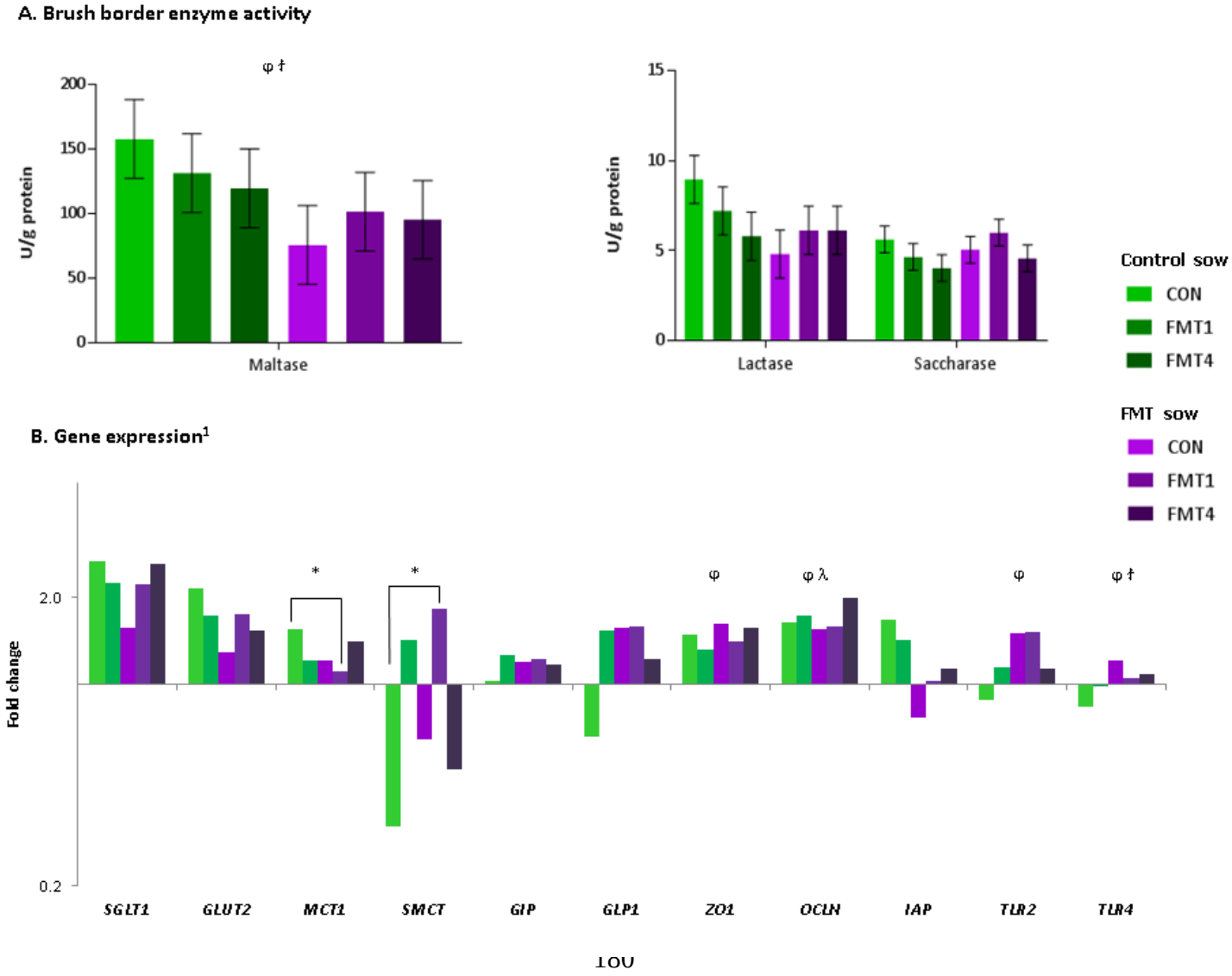

A. Brush border enzyme activity
φ ŧ
U/g protein
200
150
100
50
0
Maltase
15
10
5
0
Lactase
Saccharase
Control sow
CON
FMT1
FMT4
FMT sow
CON
FMT1
FMT4
B. Gene expression[1]
Fold change
2.0
0.2
*
*
φ
φ λ
φ
φ ŧ
SGLT1
GLUT2
MCT1
SMCT
GIP
GLP1
ZO1
OCLN
IAP
TLR2
TLR4

Figure 4.7. Effect of faecal microbiota transplantation (FMT) in sows and/or offspring on A. brush border enzyme activity and B. expression of 11 selected genes in the duodenal mucosa of offspring

*Indicates significant differences at sow × offspring treatment level ($P<0.05$); † indicates tendency for differences at sow × offspring treatment level ($P\leq0.10$); φ indicates sow treatment effect ($P\leq0.05$); λ indicates offspring treatment effect ($P\leq0.05$).

Data from 36 pigs: Sow treatment level control (CON) n=18; FMT procedure (FMTP) n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12.

[1]Bars represent log_{10}-fold changes relative to Control sow × Control offspring after normalization to Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), Beta-actin (*ACTB*) and Beta-2 microglobulin (*B2M*) gene expression.

Candidate genes measured: sodium-dependent glucose transporter 1 (*SGLT1*), monocarboxylate transporter 1 (*MCT1*), sodium-coupled monocarboxylate transporter (SMCT), intestinal alkaline phosphatase (*ALPi*), tight-junction proteins [zona occludens 1 (*ZO1*) and occludin (*OCLN*)], toll-like receptor 2 (*TLR2*) and 4 (*TLR4*), facilitated glucose transporter member 2 (*GLUT*2), glucose-dependent insulinotropic peptide (*GIP*) and glucagon-like peptide-1 (*GLP1*)

.

4.8. Supplementary Information

Table S4.1. Details on pigs removed from the study

Treatment[1]	Number of pigs	Time point/Location	Cause of death/reason for removal
CON/CON	2	Farrowing house	Crushed by sow
CON/FMT1	4		
CON/FMT4	1		
FMTP/CON	4		
FMTP/FMT1	1		
FMTP/FMT4	2		
CON/CON	1		Unknown[2]
CON/FMT1	1		
CON/FMT4	1		
FMTP/FMT4	1		
CON/CON	2		Poor growth[3]
CON/FMT1	1		
CON/FMT4	2		
FMTP/CON	1		
FMTP/FMT1	2		
FMTP/FMT4	2		
CON/FMT1	1		Haemorrhagic lesions on stomach[4]
CON/FMT1	1		Broken legs[5]
FMTP/FMT1	1		
CON/CON	1	Weaner house	Died[6]
CON/FMT1	1	Finisher house	Poor growth
CON/FMT4	1		Lameness
FMTP/CON	1		
FMTP/CON	1		Navel rupture
FMTP/CON	1		Pneumonia
FMTP/FMT1	2		Navel rupture
FMTP/FMT4	2		

[1]CON/CON: Control sow × control offspring; CON/FMT1: Control sow × FMT1 offspring; CON/FMT4: Control sow ×FMT4 offspring; FMTP/CON: FMT procedure sow × control offspring; FMTP/FMT1: FMTP sow × FMT1 offspring; FMTP/FMT4: FMTP sow × FMT4 offspring; [2] Unknown: normal organs on autopsy; [3]Euthanized due to poor growth, [4]Euthanized due to haemorrhagic lesions on stomach (possibly due to sow crushing), haematoma on right kidney and mild pneumonia symptoms, [5]Euthanized due to broken legs (caused by sow crushing); [6]Died; post-mortem showed haemorrhage in intestine and spleen.

Table S4.2. Composition of all diets used in the study (g/kg)

Diet type	Starter	Link	Weaner	Finisher	Pregnant	Lactation
Barley	0.0	0.0	24.8	38.5	89.7	34.9
Wheat	22.2	39.9	43.1	40.4	0.0	43.2
Maize	8.0	0.0	0.0	0.0	0.0	0.0
Soya	16.9	22.9	20.0	17.5	7.0	15.0
Full fat soya	10.0	7.0	5.0	0.0	0.0	0.0
Lactofeed 70[1]	20.0	20.0	0.0	0.0	0.0	0.0
Skim milk powder	12.5	5.0	0.0	0.0	0.0	0.0
Soya oil	7.5	2.5	4.0	10.0	10.0	40.0
Lysine HCl (78.8)	0.5	0.4	0.5	4.0	1.0	3.5
DL-Methionine	0.3	0.2	0.2	1.0	1.0	1.0
L-Threonine (98)	0.2	0.2	0.2	1.5	0.0	1.0
L-Tryptophan	0.09	0.05	0.02	0.0	0.0	0.0
Vitamin and mineral mix	0.3	0.3	0.3	1.0	1.5	1.5
Natuphos 5000 FTU/g[5]	0.01	0.01	0.01	0.1	0.1	0.1
Salt feed grade	0.3	0.3	0.3	3.0	4.0	4.0
Dicalcium phosphate	0.1	0.1	0.6	2.0	5.0	5.0
Limestone flour	1.1	1.1	0.9	13.0	11.0	12.0
Chemical analysis (g/kg dry matter)						
Crude protein	212	205	180	161	119	148
Crude fibre	17	18	25	24	32	25
Crude ash	56	55	44	41	37	41
Ether extract	53	52	58	27	29	63
Digestible energy (MJ/kg)[6]	17.1	17	16.9	16.3	15.6	16.8
Net energy (MJ/kg)[6]	11.4	10.3	10.6	9.8	9.5	10.5
Amino acids (g/kg)						
Lysine	15.60	14.00	13.00	11.50	6.50	9.90
Methionine	5.50	5.40	4.20	3.70	2.00	3.40
Methionine + cysteine	9.50	9.20	8.10	7.40	5.00	6.80
Threonine	10.10	9.20	8.70	7.90	4.60	6.70
Tryptophan	2.10	2.60	1.80	1.50	1.20	1.40

[1]Lactofeed 70 contains 70% lactose, 11.5% protein, 0.5% oil, 7.5% ash and 0.5% fibre (Volac, Cambridge, UK).

[2]Premix provided per kg of complete diet: Cu, 155 mg; Fe, 90 mg; Mn, 47 mg; Zn, 120 mg, I, 0.6 mg; Se, 0.3 mg; vitamin A, 6000 IU; vitamin $D_{3,}$ 1000 IU; vitamin E, 100 IU; vitamin K, 4 mg; vitamin $B_{12,}$ 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin $B_{1,}$ 2 mg; vitamin $B_{6,}$ 3 mg; Endox, 60 g.

[3]Premix provided per kg of complete diet: Cu, 15 mg; Fe, 24 mg; Mn, 31 mg; Zn, 80 mg, I, 0.3 mg; Se, 0.2 mg; vitamin A, 2000 IU; vitamin $D_{3,}$ 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin $B_{12,}$ 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin $B_{1,}$ 2 mg; vitamin $B_{6,}$ 3 mg.

[4] Premix provided per kg of complete diet: Cu, 15 mg; Fe, 70 mg; Mn, 62 mg; Zn, 80 mg, I, 0.6 mg; Se, 0.2 mg; vitamin A, 1000 IU; vitamin $D_{3,}$ 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; vitamin $B_{12,}$ 15 µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; Biotin, 200 mg; Folic acid, 5 g; vitamin $B_{1,}$ 2 mg; vitamin $B_{6,}$ 3 mg.

[5]Phytase; 5000 FTU/g equal to 500 FTU per kg finished feed.

[6]Digestible energy and net energy were calculated from book values.

Table S4.3. Forward and reverse primers used for quantitative PCR, PCR efficiency, and coefficient correlation of standard curves

Gene symbol[1]	Accession number[2]	Gene name	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)	Ref[3]	Eff. (%)[4]	Corr.[5]
ACTB	XM_003357928.2	Beta-actin	GGGCATCCTGACCCTCAAG	TGTAGAAGGTGTGATGCCAGATCT	89	1	97.3	0.99
B2M	NM_213978.1	Beta-2-microglobulin	CCCCCGAAGGTTCAGGTT	GCAGTTCAGGTAATTTGGCTTTC	66	1	102.2	0.99
GAPDH	NM_001206359.1	Glyceraldehyde-3-phosphate dehydrogenase	GGCGTGAACCATGAGAAGTATG	GGTGCAGGAGGCATTGCT	60	1	96.5	0.99
HPRT1	NM_001032376.2	Hypoxanthine guanine phosphoribosyl transferase	AGAAAAGTAAGCAGTCAGTTTCATATCAGT	ATCTGAACAAGAGAGAAAATACAGTCAATAG	131	1	92.1	0.99
OAZ1	NM_001122994.2	Ornithine decarboxylase antizyme 1	TCGGCTGAATGTAACAGAGGAA	GAGCCTGGATTGGACGTTTAAA	70	1	99.2	0.99
OCLN	NM_001163647.2	Occludin	TTGTGGGACAAGGAACGTATTTA	TGCCTGCCGACACGTTT	76	1	95.4	0.98
ZO1	XM_013993251.1	Zona occludin 1	AAGCCCTAAGTTCAATCACAATCT	ATCAAACTCAGGAGGCGGC	131	1	109.2	0.98
SGLT1 (SLC5A1)	NM_001164021.1	Sodium-dependent glucose transporter 1	TGTCTTCCTCATGGTGCCAA	AGGAGGGTCTCAGGCCAAA	149	1	108.0	0.99
GLUT2 (SLC2A2)	NM_001097417.1	Facilitated glucose transporter member 2	TACGGCATCTGCTAGCCTCAT	CCACCAATTGCAAAGATGGAC	66	2	89.3	1.00
MCT1 (SLC16A1)	AM286425.1	Monocarboxylate transporter 1	GGTGGAGGTCCTATCAGCAG	AAGCAGCCGCCAATAATCAT	74	1	96.4	1.00
SMCT (SLC5A12)	XM_003122908.1	Sodium-coupled monocarboxylate cotransporter	AGGTCTACCGCTTTGGAGCAT	GAGCTCTGATGTGAAGATGATGACA	77	2	82.3	0.99

Table S4.3. Forward and reverse primers used for quantitative PCR, PCR efficiency, and coefficient correlation of standard curves

(continued)

Gene symbol[1]	Accession number[2]	Gene name	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)	Ref[3]	Eff. (%)[4]	Corr.[5]
GIP	NM_001287408.1	Glucose-dependent insulinotropic peptide	GGATGGTGGAGCAGTTGGA	CCAATCCTGAGCTGGGTTTG	71	2	98.1	0.99
GLP1	NM_001256594.1	Glucagon-like peptide-1	GCTGATGGTGGCGATCTTGT	TCCCAGCTCTTCCGAAACTC	69	2	98.1	0.99
TRL2	NM_213761.1	Toll-like receptor 2	AATAAGTTGAAGACGCTCCCAGAT	GTTGCTCCTTAGAGAAAGTATTGATCGT	97	1	92.7	0.99
TRL4	AB188301.2	Toll-like receptor 4	TGTGGCCATCGCTGCTAAC	GGTCTGGGCAATCTCATACTCA	124	1	105.8	0.98
ALPI	XM_003133729.3	Intestinal alkaline phosphatase	AGGAACCCAGAGGGACCATTC	CACAGTGGCTGAGGGACTTAGG	83	2	97.1	0.99

[1]Gene symbol: Alternate gene names are shown in brackets.
[2]Accession number: National Center for Biotechnology Information (NCBI) Entrez Gene (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene).
[3]Ref: references for oligonucleotide primer sequences. 1) Metzler-Zebeli BU, Mann E, Ertl R, Schmitz-Esser S, Wagner M, Klein D, Ritzmann M, Zebeli Q. Dietary calcium concentration and cereals differentially affect mineral balance and tight junction proteins expression in jejunum of weaned pigs. Br J Nutr. 2015; 113(7):1019-31. doi: 10.1017/S0007114515000380.; 2) Metzler-Zebeli BU, Ertl R, Grüll D, Molnar T, Zebeli Q. Enzymatically modified starch up-regulates expression of incretins and sodium-coupled monocarboxylate transporter in jejunum of growing pigs. Animal 2016; 11(7):1180-1188. Doi: 10.1017/S175131116002615.
[4]Eff: PCR efficiency: E = 10(-1/slope)-1.
[5]Corr: Correlation coefficient of standard curve.

Table S4.4. Significant sow x offspring treatment interactions for all parameters measured in the study[1]

Sow treatment	Control[2]			FMTP[3]			S.E.M.	P-value		
Offspring treatment	**Control[4]**	**FMT1[5]**	**FMT4[6]**	**Control**	**FMT1**	**FMT4**		**Interaction**	**Sow**	**Offspring**
Body weight (Kg)										
Day 70	35.2^{a}	29.9^{b}	30.4^{b}	$29.1^{b,c}$	$27.1^{c,d}$	26.4^{d}	2.29	0.01	0.02	0.19
Day 155	127.9^{a}	116.0^{c}	120.9^{b}	115.3^{c}	114.2^{c}	111.3^{c}	2.87	<0.001	<0.001	<0.001
Residual Feed Intake (g/day)										
Weaning to Day 155	21.2^{A}	-30.2^{B}	24.6^{A}	-30.1^{B}	$9.4^{A,B}$	$1.33^{A,B}$	21.38	0.08	0.51	0.52
Alpha diversity (Shannon index)										
Faeces day 50	3.49^{b}	3.21^{b}	$3.73^{a,b}$	$3.70^{a,b}$	4.13^{a}	4.22^{a}	0.226	0.02	0.004	0.22
Ileum	1.80^{b}	2.74^{a}	1.84^{b}	1.71^{b}	1.83^{b}	1.71^{b}	0.123	<0.001	<0.001	<0.001
Bacterial load (log_{10} copies of 16S rRNA gene/ng DNA)										
Ileum	$4.35^{a,b}$	$3.97^{a,b}$	3.91^{b}	4.53^{a}	$3.99^{a,b}$	$4.04^{a,b}$	0.115	<0.001	0.28	<0.001

Table S4.4. Significant sow x offspring treatment interactions for all parameters measured in the study[1] (continued)

Sow treatment	Control[2]			FMTP[3]			S.E.M.	P-value		
Offspring treatment	Control[4]	FMT1[5]	FMT4[6]	Control	FMT1	FMT4		Interaction	Sow	Offspring
Microbiota composition [median relative abundance (%)]										
Faeces at weaning										
G_*Asteroleplasma*	0.51^{b}	0.57^{b}	0.17^{b}	2.09^{a}	$1.76^{a,b}$	$1.89^{a,b}$	0.06	<0.001	<0.001	0.34
G_*Blautia*	$1.46^{a,b}$	$1.00^{a,b}$	0.45^{b}	$0.59^{a,b}$	$1.67^{a,b}$	3.32^{a}	0.22	0.008	0.06	0.59
G_*Faecalibacterium*	0.54^{c}	1.09^{a}	0.74^{a}	0.38^{b}	0.19^{b}	0.82^{a}	0.10	0.05	0.02	0.29
G_*Oribacterium*	1.58^{a}	1.39^{a}	5.04^{a}	0.38^{b}	0.14^{b}	1.04^{a}	0.07	<0.001	<0.001	0.14
P_*Planctomycetes*	2.72^{a}	0.37^{b}	$1.43^{a,b}$	$1.52^{a,b}$	0.39^{b}	$0.83^{a,b}$	0.16	0.009	0.29	0.001
Faeces at day 50										
P_*Actinobacteria*	$0.10^{a,b}$	0.05^{b}	$0.11^{a,b}$	$0.13^{a,b}$	$0.27^{a,b}$	1.08^{a}	0.015	<0.001	<0.001	0.04
G_*Anaerostipes*	$0.021^{a,b}$	0.002^{b}	$0.189^{a,b}$	$0.019^{a,b}$	$0.056^{a,b}$	0.451^{a}	0.004	0.003	0.06	0.002
G_*Asteroleplasma*	$0.90^{a,b}$	$0.33^{a,b}$	$0.64^{a,b}$	0.07^{b}	$0.45^{a,b}$	1.05^{a}	0.026	0.002	0.14	0.06
G_*Campylobacter*	$0.76^{a,b}$	0.22^{b}	$0.54^{a,b}$	$0.96^{a,b}$	1.44^{a}	1.43^{a}	0.104	0.003	0.004	0.37
G_*Alistipes*	0.27^{b}	0.14^{b}	0.18^{b}	$0.33^{a,b}$	0.59^{a}	0.70^{a}	0.066	0.01	<0.001	0.85
G_*Peptococcus*	0.22^{b}	0.08^{b}	0.12^{b}	0.12^{b}	0.14^{b}	2.13^{a}	0.033	<0.001	0.01	0.003
P_*Tenericutes*	$0.90^{a,b}$	$0.33^{a,b}$	$0.64^{a,b}$	0.07^{b}	$0.46^{a,b}$	1.04^{a}	0.025	0.001	0.89	0.59
Faeces at day 65										
G_*Alistipes*	$0.32^{a,b}$	0.16^{b}	0.47^{a}	0.68^{a}	0.55^{a}	0.14^{b}	0.072	0.005	0.34	0.22
G_*Anaerostipes*	0.51^{s}	$0.16^{s,b}$	0.65^{a}	0.02^{b}	0.84^{a}	0.24^{a}	0.005	<0.001	0.07	0.06
G_*Campylobacter*	0.37^{b}	0.39^{b}	$0.98^{a,b}$	1.62^{a}	1.47^{a}	$0.79^{a,b}$	0.182	0.002	0.01	0.88
G_*Terrisporobacter*	0.13^{a}	0.03^{a}	0.12^{a}	0.07^{a}	0.01^{b}	0.01^{b}	0.001	<0.001	0.008	0.008
G_*Peptococcus*	0.22^{b}	0.08^{b}	0.12^{b}	0.12^{b}	0.14^{b}	2.13^{a}	0.033	0.001	0.007	0.001
G_*Ruminococcus*	$0.30^{a,b}$	$0.31^{a,b}$	$0.24^{a,b}$	1.11^{a}	$0.47^{a,b}$	0.13^{b}	0.067	0.001	0.17	0.003

Table S4.4. Significant sow x offspring treatment interactions for all parameters measured in the study[1] (continued)

Sow treatment	Control[2]			FMTP[3]			S.E.M.	P-value		
Offspring treatment	Control[4]	FMT1[5]	FMT4[6]	Control	FMT1	FMT4		Interaction	Sow	Offspring
Microbiota composition [median relative abundance (%)]										
Faeces at day 100										
G_*Peptococcus*	0.13^{a,b}	0.06^{b}	0.25^{a}	0.27^{a}	0.15^{a,b}	0.84^{a}	0.057	<0.001	0.65	<0.001
Ileum										
P_*Actinobacteria*	0.07^{b}	0.64^{a,b}	0.85^{a}	0.10^{a,b}	0.17^{a,b}	0.04^{b}	0.015	<0.001	0.001	0.02
G_*Blautia*	0.09^{b}	1.97^{a}	0.09^{b}	0.01^{c}	0.11^{b}	0.12^{b}	0.005	<0.001	<0.001	<0.001
G_*Butyricicoccus*	0.064^{a}	0.368^{a}	0.062^{a}	0.005^{b}	0.234^{a}	0.096^{a}	0.0023	<0.001	0.55	0.84
G_*Butyricimonas*	0.05^{b}	0.89^{a}	0.03^{b}	0.04^{b}	0.09^{b}	0.05^{b}	0.018	<0.001	0.006	<0.001
P_*Spirochaetaes*	11.41^{a,b}	19.41^{a,b}	7.27^{b}	24.07^{a}	10.31^{a,b}	9.40^{a,b}	4.680	0.004	0.52	0.01
P_*Chlamydiae*	0.18^{a,b}	4.30^{a}	0.14^{a,b}	0.04^{b}	0.38^{a,b}	0.22^{a,b}	0.020	<0.001	<0.001	<0.001
G_*Chlamydia*	0.18^{b,c}	4.29^{a}	0.14^{b,c}	0.04^{c}	0.38^{b}	0.22^{b,c}	0.021	<0.001	0.17	0.35
G_*Faecalibacterium*	0.04^{a,b}	0.21^{a}	0.03^{a,b}	0.01^{b}	0.09^{a,b}	0.05^{a,b}	0.005	0.002	0.14	0.002
G_*Oscillibacter*	0.05^{c}	0.07^{c}	0.23^{b}	1.63^{a}	0.79^{a}	0.18^{b}	0.022	<0.001	<0.001	0.70
G_*Prevotella*	8.62^{a,b}	4.31^{b}	5.82^{b}	10.93^{a,b}	9.12^{a,b}	19.02^{a}	2.281	0.03	0.005	0.20
G_*Sphaerochaeta*	5.52^{b}	8.99^{a,b}	1.80^{b}	17.19^{a}	3.76^{b}	5.71^{b}	0.821	0.01	0.17	0.04
Predicted microbial function in the ileum [median relative abundance (%)]										
Alpha-linolenic acid	0.51^{b}	5.79^{a}	5.41^{a}	5.61^{a}	0.91^{b}	0.63^{b}	0.174	<0.001	0.03	0.56
Tryptophan metabolism	3.02^{a,b}	3.87^{a}	2.45^{b}	3.86^{a,b}	2.97^{a,b}	2.74^{a,b}	2.072	0.002	0.67	0.004
Protein kinases	3.06^{a,b}	3.76^{a}	2.57^{b}	3.44^{a,b}	3.17^{a,b}	2.83^{a,b}	2.299	<0.001	0.79	<0.001
Pentose and glucuronate interconversions	3.09^{a,b}	3.19^{a,b}	2.28^{b}	4.05^{a}	3.09^{a,b}	3.22^{a,b}	1.936	<0.001	0.008	0.88
Propanoate metabolism	3.19^{a,b}	3.14^{a,b}	2.62^{b}	3.58^{a}	3.31^{a,b}	2.93^{a,b}	0.387	<0.001	0.57	0.001
Primary and secondary bile acid biosynthesis	2.93^{b}	7.30^{a}	2.97^{b}	1.48^{b}	2.84^{b}	1.29^{b}	0.842	<0.001	<0.001	<0.001

Table S4.4. Significant sow x offspring treatment interactions for all parameters measured in the study[1] (continued)

Sow treatment	Control[2]			FMTP[3]			S.E.M.	P-value		
Offspring treatment	**Control[4]**	**FMT1[5]**	**FMT4[6]**	**Control**	**FMT1**	**FMT4**		**Interaction**	**Sow**	**Offspring**
Predicted microbial function in the ileum [median relative abundance (%)]										
Steroid biosynthesis	1.23^{b}	6.43^{a}	7.14^{a}	$3.89^{a,b}$	$2.34^{a,b}$	1.27^{b}	0.579	<0.001	<0.001	<0.001
Pentose phosphate pathway	$3.11^{a,b}$	3.35^{a}	2.66^{b}	3.60^{a}	$3.12^{a,b}$	$2.98^{s,b}$	0.448	<0.001	0.08	0.001
Pyruvate metabolism	$3.07^{a,b}$	3.46^{a}	2.49^{b}	3.71^{a}	$3.06^{a,b}$	$3.07^{a,b}$	2.224	0.001	0.07	0.005
Tetracycline biosynthesis	$2.80^{a,b}$	4.21^{a}	1.86^{b}	4.34^{a}	$2.96^{a,b}$	$2.85^{a,b}$	1.490	<0.001	0.08	<0.001
Styrene degradation	1.46^{b}	6.22^{a}	1.82^{b}	$2.07^{a,b}$	$2.40^{a,b}$	5.16^{a}	1.376	<0.001	0.38	<0.001
Various types of N-glycan biosynthesis	1.32^{c}	0.66^{c}	22.34^{a}	15.48^{a}	5.01^{b}	1.33^{c}	0.306	<0.001	0.06	0.006
Stilbenoid, diarylheptanoid and gingerol biosynthesis	2.10^{b}	1.57^{b}	2.36^{b}	38.42^{a}	1.57^{b}	1.57^{b}	0.684	<0.001	0.64	<0.001
Volatile fatty acids (µmol/g digesta)										
Ileum										
Propionic acid	$26.8^{a,b}$	19.9^{b}	14.9^{b}	18.9^{b}	42.7^{a}	$27.7^{a,b}$	8.25	0.02	0.03	0.33
Butyric acid	7.1^{b}	33.9^{a}	23.8^{a}	13.5^{b}	11.9^{b}	13.7^{b}	4.29	0.001	0.12	0.01
Isovaleric acid	$16.7^{a,b}$	$13.1^{a,b}$	24.0^{a}	$14.7^{a,b}$	20.2^{a}	8.3^{b}	5.63	0.03	0.37	0.91
Intestinal histology										
Duodenum										
Goblet cell (number per villi)	41^{a}	$36^{a,b}$	39^{a}	34^{b}	34^{b}	33^{b}	1.8	0.05	0.004	0.63
Ileum										
Villus height (µm)	$545^{a,b}$	602^{a}	$585^{a,b}$	$452^{a,b}$	413^{b}	$443^{a,b}$	33.1	0.001	0.05	0.51
Gene expression (Fold change[7])										
MCT1	$0.00^{a,b}$	0.24^{a}	$0.08^{a,b}$	$0.08^{a,b}$	0.03^{b}	$0.17^{a,b}$	-	0.04	0.91	0.17
SMCT	$0.00^{a,b}$	-0.22^{b}	$0.06^{a,b}$	$-0.14^{a,b}$	0.16^{a}	$0.17^{a,b}$	-	0.003	0.78	0.74
Haematology										
Monocyte (no. x 10^3 cells/µL)	0.52^{b}	$0.78^{a,b}$	$0.77^{a,b}$	0.83^{a}	$0.66^{a,b}$	$0.68^{a,b}$	0.083	0.02	0.59	0.79
Red cell distribution width (fL)	$19.4^{a,b}$	20.4^{a}	18.5^{b}	$19.4^{a,b}$	18.7^{b}	$19.1^{a,b}$	0.44	0.04	0.37	0.19

[1]Least squares means and pooled standard error of the mean is presented.

Sows were assigned to one of two treatment groups: [2]Control Control (n=9) and [3]FMT procedure (FMTP; n=9); FMT sows received FMT via gastric intubation on days 70 and 100 of gestation. Piglets were assigned to one of three treatment groups at birth: [4]Control; [5]FMT1 (FMT at birth) and [6]FMT4 (FMT at birth, days 3, 7 and 28 of age).Data from 36 pigs: Sow treatment level control n=18; FMTP n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12. [7]Values represent $\log_{10}$-fold changes relative to Control sow x Control offspring after normalization to Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), Beta-actin (*ACTB*) and Beta-2 microglobulin (*B2M*) gene expression.

[a,b,c] Within each row, values that do not share a common superscript are significantly different due to a sow × offspring treatment interaction ($P \leq 0.05$).

Table S4.5. Relative abundance (%) of bacterial genera that differed between sow treatments pre- and post-antibiotic treatment

Genus (Phylum)	CON[1]	FMTP[2]	Confidence interval		P-value
			Lower	Upper	
Pre-antibiotic faeces					
Butyricimonas (*Bacteroidetes*)	0.06	0.16	0.036	0.282	0.03
Fusobacterium (*Fusobacteria*)	0.12	0.04	0.025	0.254	0.04
Roseburia (*Firmicutes*)	0.22	0.02	0.002	0.848	0.02
Schwartzia (*Firmicutes*)	0.95	0.19	0.048	0.395	0.02
Post-antibiotic faeces					
Asteroleplasma (*Tenericutes*)	0.3	2.5	0.17	4.27	<0.0001
Butyricicoccus (*Firmicutes*)	0.1	1.1	0.02	6.19	0.003
Butyricimonas (*Bacteroidetes*)	0.1	0.2	0.04	0.41	0.03
Chlamydia (*Chlamydiae*)	0.8	0.1	0.02	4.06	0.01
Citrobacter (*Proteobacteria*)	0.03	0.29	0.016	1.069	0.004
Desulfovibrio (*Proteobacteria*)	0.1	0.9	0.06	2.23	0.0002
Eubacterium (*Firmicutes*)	0.12	0.01	0.001	0.239	0.001
Faecalibacterium (*Firmicutes*)	0.82	0.01	0.003	1.326	<0.0001
Fibrobacter (*Fibrobacteres*)	0.044	0.003	0.0006	0.0917	0.002

Table S4.5. Relative abundance (%) of bacterial genera that differed between sow treatments pre- and post-antibiotic treatment (continued)

Genus (Phylum)	CON[1]	FMTP[2]	Confidence interval		P-value
			Lower	Upper	
Fusicatenibacter (*Firmicutes*)	0.03	0.01	0.001	0.069	0.02
Gemmiger (*Proteobacteria*)	0.06	0.01	0.001	0.147	0.05
Lachnospiraceae Incertae Sedis (*Firmicutes*)	2.7	0.5	0.25	4.15	<0.0001
Lactobacillus (*Firmicutes*)	0.3	2.6	0.16	5.85	<0.0001
Oribacterium (*Firmicutes*)	0.5	1.7	0.24	3.55	0.01
Oscillibacter (*Firmicutes*)	1.95	0.04	0.009	4.391	<0.0001
Ruminococcus (*Firmicutes*)	0.5	16.7	0.2	29.39	<0.0001
Ruminococcus2 (*Firmicutes*)	1.8	0.4	0.21	3.43	0.003
Selenomonas (*Firmicutes*)	1.58	0.01	0.002	3.111	<0.0001
Sphaerochaeta (*Spirochaetes*)	2.6	11.5	1.17	25.83	0.01
Succinivibrio (*Proteobacteria*)	0.2	1.6	0.13	3.25	0.0003
Unclassified (*Grouped*[3])	42.3	27.4	22.12	52.24	0.006
Veillonella (*Firmicutes*)	0.5	0.1	0.06	0.94	0.03
Victivallis (*Lentisphaerae*)	0.97	0.01	0.001	1.886	<0.0001

Sows were assigned to one of two treatment groups: [1]Control (CON; n=9) and [2]FMT procedure (FMTP; n=9). FMT sows received FMT via gastric intubation on days 70 and 100 of gestation; [3]Grouped: all bacterial taxa "unclassified" at genus level were grouped together for statistical analysis. Data from 12 sows: control (CON) n=6; FMT n=6.

Table S4.6. Effect of faecal microbiota transplantation (FMT) on serum biochemical parameters in offspring at ~155 days of age[1]

Sow treatment	Control[2]			FMTP[3]			S.E.M.	P-value		
Offspring treatment	Control[4]	FMT1[5]	FMT4[6]	Control	FMT1	FMT4		Interaction	Sow	Offspring
Total protein (g/L)	69.6	67.1	64.9	69.8	64.3	64.7	1.94	0.71	0.58	0.04
Blood urea nitrogen (mg/dL)	19.9	17.8	24.9	20.2	24.4	20.9	2.85	0.19	0.69	0.61
Glucose (mmol/L)	5.67	6.05	6.77	5.90	6.73	6.98	0.532	0.88	0.40	0.14
Triglycerides (mmol/L)	0.64	0.64	0.59	0.72	0.61	0.64	0.061	0.67	0.52	0.49
Cholesterol (mmol/L)	2.83	2.89	3.53	2.71	2.47	2.56	0.295	0.37	0.04	0.44
Creatinine (µmol/L)	150	141	151	157	137	143	9.74	0.71	0.81	0.36
Creatine kinase (µmol/L)	159	107	158	176	200	144	30.42	0.22	0.21	0.84

[1]Least squares means and pooled standard error of the mean is presented.

Sows were assigned to one of two treatment groups: [2]Control Control (n=9) and [3]FMT procedure (FMTP; n=9); FMT sows received FMT via gastric intubation on days 70 and 100 of gestation. Piglets were assigned to one of three treatment groups at birth: [4]Control; [5]FMT1 (FMT at birth) and [6]FMT4 (FMT at birth, days 3, 7 and 28 of age).

Data from 36 pigs: Sow treatment level control n=18; FMTP n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12. [a,b,c] Within each row, values that do not share a common superscript are significantly different ($P \leq 0.05$).

Table S4.7. Effect of faecal microbiota transplantation (FMT) on haematological parameters in offspring at ~155 days of age[1]

Sow treatment	Control[2]			FMT[3]			S.E.M.	P-value		
Offspring treatment	Control[4]	FMT1[5]	FMT4[6]	Control	FMT1	FMT4		Interaction	Sow	Offspring
White blood cells (x 10^3 cells/µL)	26	36	27	29	27	30	3.4	0.12	0.76	0.39
Lymphocytes										
%	27	23	31	27	26	27	2.2	0.21	0.95	0.13
no. x 10^3 cells/µL	6.87	7.28	8.16	7.57	7.14	7.72	0.498	0.49	0.93	0.25
Monocytes										
%	2.1	2.4	3.0	3.0	2.5	2.4	0.42	0.17	0.71	0.83
no. x 10^3 cells/µL	0.52^{b}	$0.78^{a,b}$	$0.77^{a,b}$	0.83^{a}	$0.66^{a,b}$	$0.68^{a,b}$	0.083	0.02	0.59	0.79
Granulocytes										
%	70	61	65	72	73	61	7.3	0.52	0.62	0.49
no. x 10^3 cells/µL	18.4	18.7	17.9	20.5	19.2	20.1	2.09	0.92	0.36	0.97
Red blood cells (x 10^6 cells/µL)	7.43	7.45	6.92	7.44	7.53	7.27	0.219	0.71	0.41	0.17
Red cell distribution width (fL)	$19.4^{a,b}$	20.4^{a}	18.5^{b}	$19.4^{a,b}$	18.7^{b}	$19.1^{a,b}$	0.44	0.04	0.37	0.19
Haemoglobin (g/dL)	13.7	13.8	13.8	13.5	13.9	13.4	0.39	0.83	0.54	0.68
Haematocrit (%)	0.43	0.45	0.41	0.42	0.44	0.41	0.015	0.92	0.59	0.18
Mean corpuscular volume (fL)	58.1	59.9	58.9	56.4	58.2	56.9	1.08	0.98	0.05	0.25
Mean corpuscular haemoglobin										
%	18.5	18.6	18.5	18.2	18.6	18.4	0.40	0.91	0.62	0.83
Pg	31.9	31.2	31.4	32.2	31.9	32.4	0.55	0.81	0.14	0.62
Platelets (x 10^6 cells /µL)	288	199	199	212	191	209	32.5	0.39	0.36	0.21
Mean platelet volume (fL)	10.03	10.02	9.65	9.07	9.32	9.33	0.327	0.60	0.02	0.87

[1]Least squares means and pooled standard error of the mean is presented.

Sows were assigned to one of two treatment groups: [2]Control Control (n=9) and [3]FMT procedure (FMTP; n=9); FMT sows received FMT via gastric intubation on days 70 and 100 of gestation. Piglets were assigned to one of three treatment groups at birth: [4]Control; [5]FMT1 (FMT at birth) and [6]FMT4 (FMT at birth, days 3, 7 and 28 of age).Data from 36 pigs Sow treatment level: control n=18; FMTP n=18. Offspring treatment level: control n=12; FMT1 n=12; FMT4 n=12.

[6]Least squares means and pooled standard error of the mean are presented.

[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P \leq 0.05$).

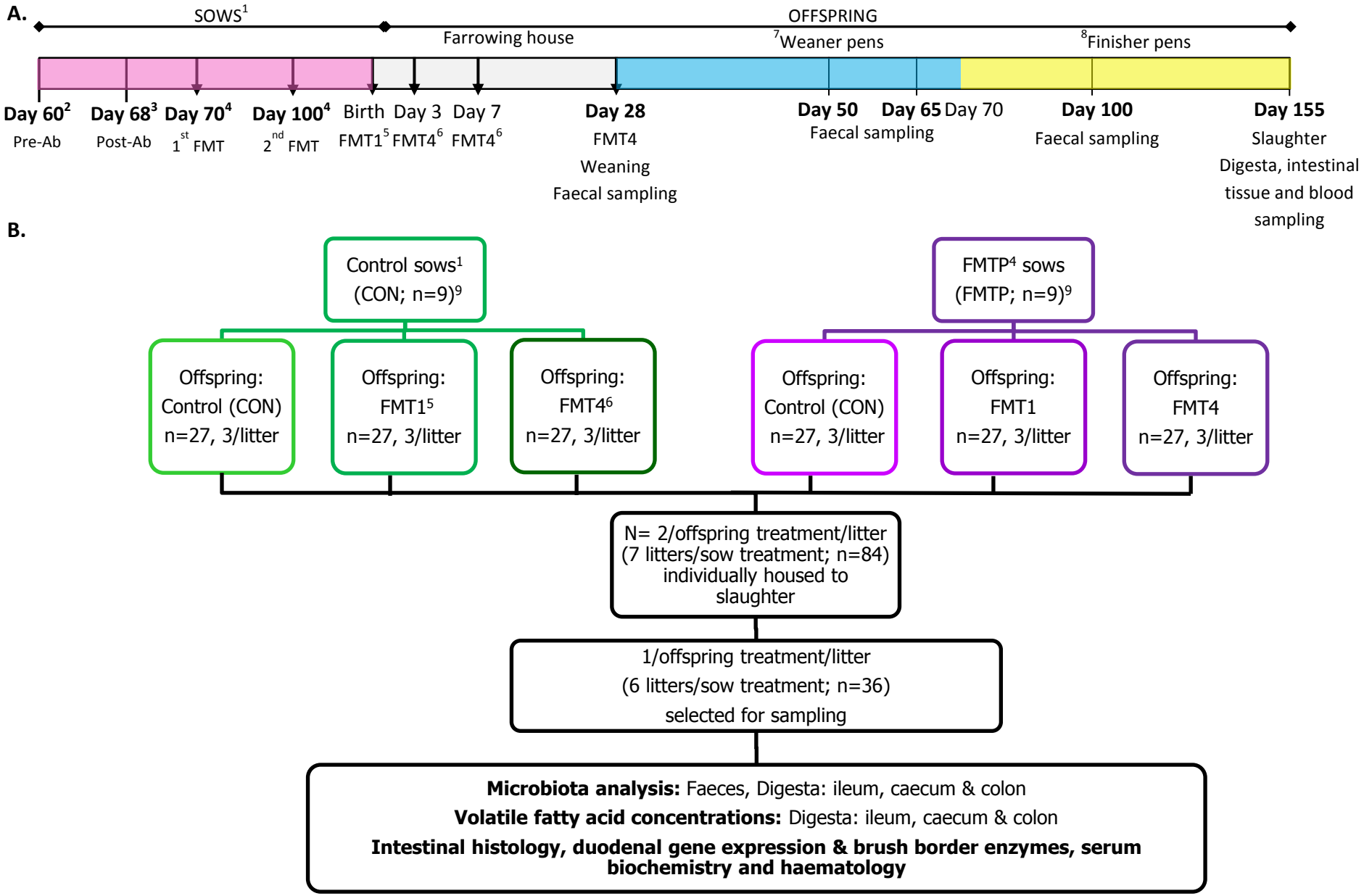

A.
SOWS[1]
OFFSPRING
Farrowing house
[7]Weaner pens
[8]Finisher pens
Day 60[2]
Pre-Ab
Day 68[3]
Post-Ab
Day 70[4]
1st FMT
Day 100[4]
2nd FMT
Birth
FMT1[5]
Day 3
FMT4[6]
Day 7
FMT4[6]
Day 28
FMT4
Weaning
Faecal sampling
Day 50
Faecal sampling
Day 65
Day 70
Day 100
Faecal sampling
Day 155
Slaughter
Digesta, intestinal tissue and blood sampling
B.
Control sows[1] (CON; n=9)[9]
FMTP[4] sows (FMTP; n=9)[9]
Offspring: Control (CON) n=27, 3/litter
Offspring: FMT1[5] n=27, 3/litter
Offspring: FMT4[6] n=27, 3/litter
Offspring: Control (CON) n=27, 3/litter
Offspring: FMT1 n=27, 3/litter
Offspring: FMT4 n=27, 3/litter
N= 2/offspring treatment/litter (7 litters/sow treatment; n=84) individually housed to slaughter
1/offspring treatment/litter (6 litters/sow treatment; n=36) selected for sampling
Microbiota analysis: Faeces, Digesta: ileum, caecum & colon
Volatile fatty acid concentrations: Digesta: ileum, caecum & colon
Intestinal histology, duodenal gene expression & brush border enzymes, serum biochemistry and haematology

Figure S4.1. Schematic overview depicting A. experiment timeline and B. study design and sample analysis

[1]Eighteen multiparous F1 sows (Large White × Landrace; Hermitage Genetics, Kilkenny, Ireland) were artificially inseminated at random using semen from one of two boars [Hylean Maxgro; Hermitage genetics; boars selected with the lowest FCE values (the most feed efficient)]. [2]Sows on FMT procedure (FMTP) treatment given a 1 week course of antibiotics from day 61 of gestation to kill off as much of the resident microbiota as possible. Antibiotic cocktail consisted of a combination of 20 mg/Kg/day Amoxicillin Trihydrate (amoxinsol®, Vetoquinol UK Ltd., Buckingham, UK), 10 mg/Kg/day lincomycin-spectinomycin (Linco-Spectin® 100, Pfizer, Cork, Ireland) and 100,000 IU/Kg/day of colistin (Coliscour®, Ceva Sante Animale, Libourne, France). [3]On day 68, to remove the feed volume and remaining microbes present in the GIT, sows received 2 doses of a purgative which consisted of 145 g Picolax® powder (Ferring Ireland Ltd., Dublin, Ireland) dissolved in 1350 mL water, providing 10 mg sodium picosulfate, 3.5 mg magnesium oxide and 12 g citric acid per dose.. Sows were then fasted for 36 h to empty the gastrointestinal tract. [4]On days 70 and 100 of gestation, each sow was given 200 mL of thawed inoculum (a dose of 2.6×10^{11} CFU) via gastric intubation 1 h after administration of a proton-pump inhibitor [7 tablets, each containing 40 mg omeprazole (Rowex Ltd., Cork, Ireland) dissolved in 400 mL lukewarm water] to reduce the likelihood of inactivation of the inoculum by gastric acid.

Piglets were given 8 mL of thawed inoculum (a dose of 9.4×10^9 CFU) orally via syringe [5]at birth, or [6]at birth and days 3, 7, and 28 of age. [7]Pigs were individually housed from weaning to 70 days of age in weaner pens (1.2 m × 0.9 m) with plastic slats (Faroex, Manitoba, Canada) and solid plastic dividers between pens. [8]From 70 to 155 days of age, pigs were housed in fully slatted finisher pens (1.81 m × 1.18 m) with solid plastic panel partitions. [9]Only 6 sows per treatment were sampled.

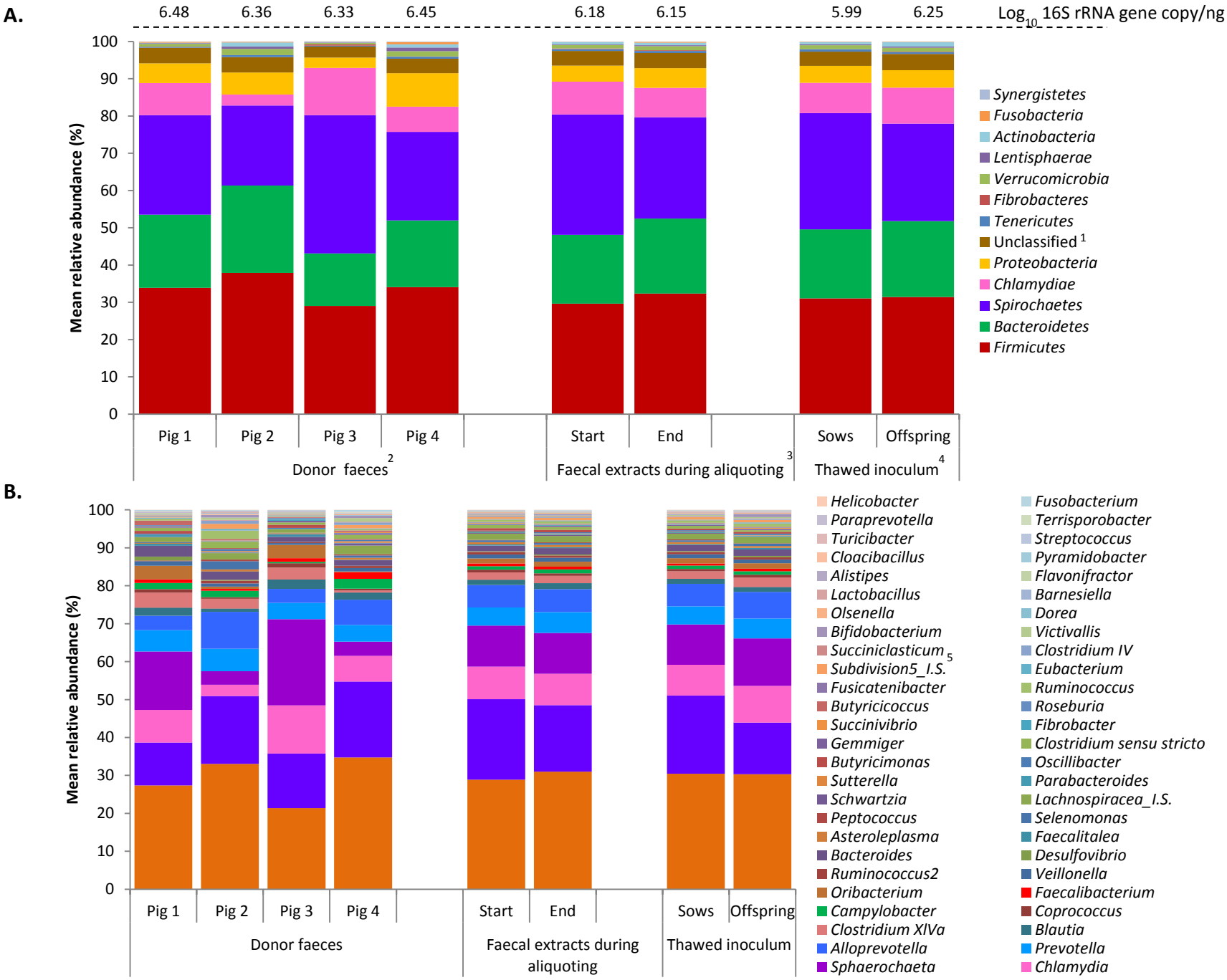

A.
6.48
6.36
6.33
6.45
6.18
6.15
5.99
6.25
Log10 16S rRNA gene copy/ng
Mean relative abundance (%)
Synergistetes
Fusobacteria
Actinobacteria
Lentisphaerae
Verrucomicrobia
Fibrobacteres
Tenericutes
Unclassified 1
Proteobacteria
Chlamydiae
Spirochaetes
Bacteroidetes
Firmicutes
Pig 1
Pig 2
Pig 3
Pig 4
Start
End
Sows
Offspring
Donor faeces 2
Faecal extracts during aliquoting 3
Thawed inoculum 4
B.
Mean relative abundance (%)
Helicobacter
Paraprevotella
Turicibacter
Cloacibacillus
Alistipes
Lactobacillus
Olsenella
Bifidobacterium
Succiniclasticum 5
Subdivision5_I.S.
Fusicatenibacter
Butyricicoccus
Succinivibrio
Gemmiger
Butyricimonas
Sutterella
Schwartzia
Peptococcus
Asteroleplasma
Bacteroides
Ruminococcus2
Oribacterium
Campylobacter
Clostridium XlVa
Alloprevotella
Sphaerochaeta
Fusobacterium
Terrisporobacter
Streptococcus
Pyramidobacter
Flavonifractor
Barnesiella
Dorea
Victivallis
Clostridium IV
Eubacterium
Ruminococcus
Roseburia
Fibrobacter
Clostridium sensu stricto
Oscillibacter
Parabacteroides
Lachnospiracea_I.S.
Selenomonas
Faecalitalea
Desulfovibrio
Veillonella
Faecalibacterium
Coprococcus
Blautia
Prevotella
Chlamydia
Pig 1
Pig 2
Pig 3
Pig 4
Start
End
Sows
Offspring
Donor faeces
Faecal extracts during aliquoting
Thawed inoculum

Figure S4.2. Total bacterial load and microbial composition at the A. phylum and B. genus level in faeces from donor pigs, and also at the start and end of aliquoting of the resultant faecal extracts used as inocula and in the thawed inocula administered to sows and offspring

[1]Unclassified: Taxonomic assignment cut off was set at > 80%. [2]Donor faeces were from low RFI pigs at 130 days of age, and mean values across inocula preparation days are presented. [3]Mean values across inocula preparation days. [4]Mean values from thawed inocula administered. [5]I.S.: *Incertae sedis*.

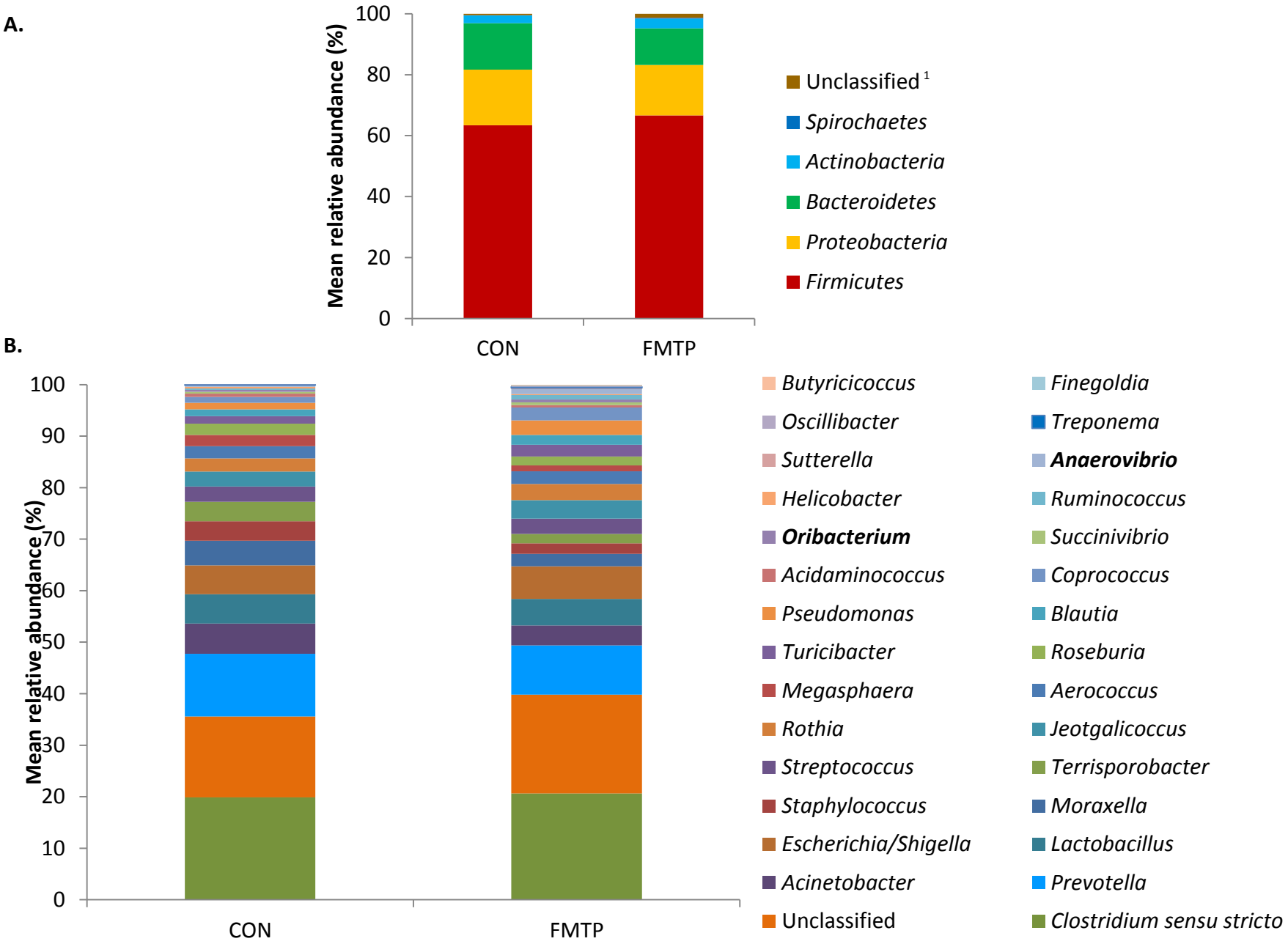

A.
Mean relative abundance (%)
100
80
60
40
20
0
CON
FMTP
Unclassified [1]
Spirochaetes
Actinobacteria
Bacteroidetes
Proteobacteria
Firmicutes
B.
Mean relative abundance (%)
100
90
80
70
60
50
40
30
20
10
0
CON
FMTP
Butyricicoccus
Oscillibacter
Sutterella
Helicobacter
Oribacterium
Acidaminococcus
Pseudomonas
Turicibacter
Megasphaera
Rothia
Streptococcus
Staphylococcus
Escherichia/Shigella
Acinetobacter
Unclassified
Finegoldia
Treponema
Anaerovibrio
Ruminococcus
Succinivibrio
Coprococcus
Blautia
Roseburia
Aerococcus
Jeotgalicoccus
Terrisporobacter
Moraxella
Lactobacillus
Prevotella
Clostridium sensu stricto

Figure S4.3. Microbial composition at the A. phylum and B. genus level in sow colostrum collected at farrowing

Data from 7 sows: Control (CON) n=4, FMT procedure (FMTP) n=3.

[1]Unclassified: Taxonomic assignment cut off was set at > 80%. Bacterial genera in bold differed significantly between CON and FMTP sows (P<0.05)

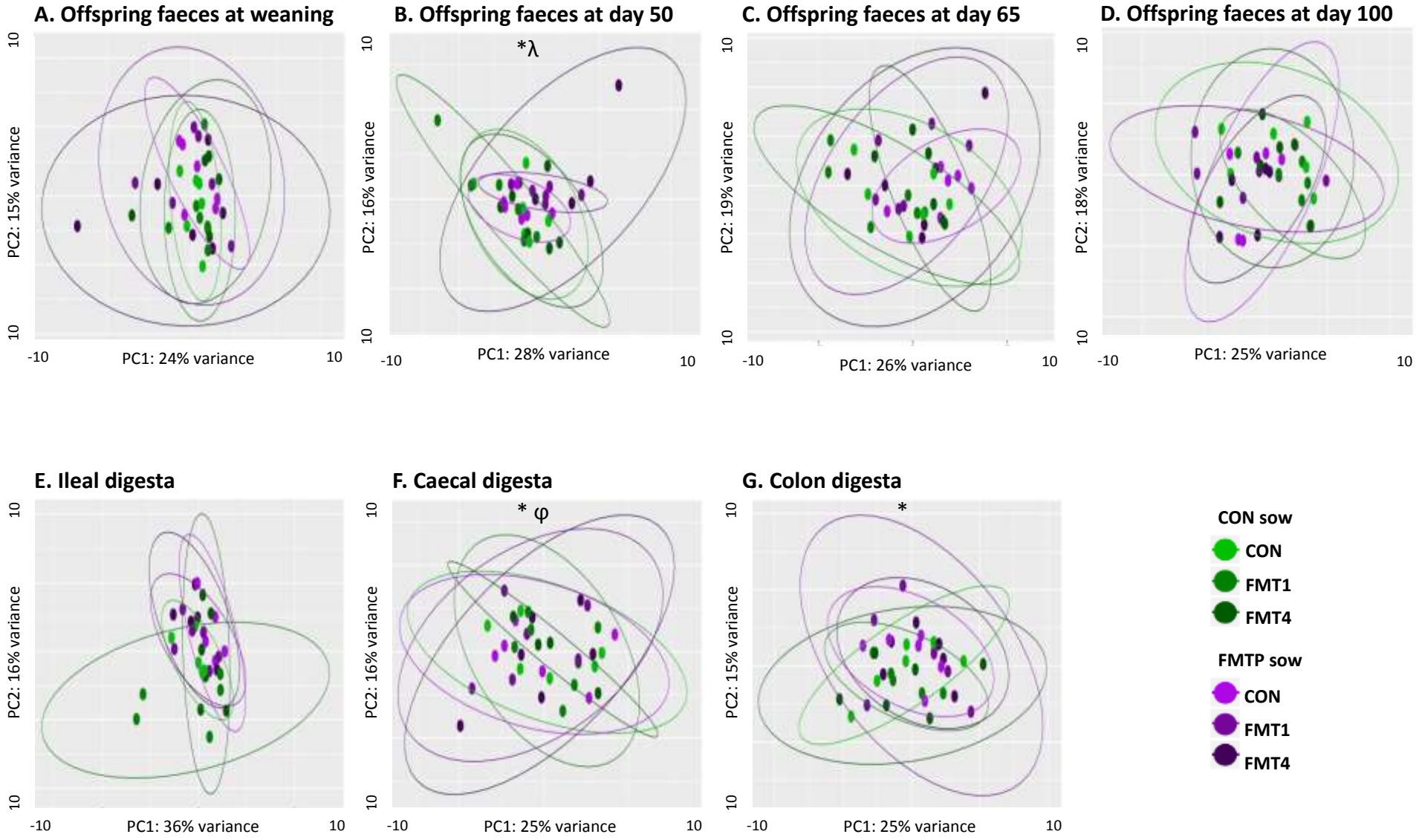

A. Offspring faeces at weaning
PC2: 15% variance
PC1: 24% variance
B. Offspring faeces at day 50
*λ
PC2: 16% variance
PC1: 28% variance
C. Offspring faeces at day 65
PC2: 19% variance
PC1: 26% variance
D. Offspring faeces at day 100
PC2: 18% variance
PC1: 25% variance
E. Ileal digesta
PC2: 16% variance
PC1: 36% variance
F. Caecal digesta
* φ
PC2: 16% variance
PC1: 25% variance
G. Colon digesta
*
PC2: 15% variance
PC1: 25% variance
CON sow
CON
FMT1
FMT4
FMTP sow
CON
FMT1
FMT4

Figure S4.4. Microbial diversity variation due to faecal microbiota transplantation (FMT) in sows and/or offspring in the offspring faeces at A. 28, B. 50, C. 65 and D. 100 days of age and in the offspring E. ileum, F. caecum and G. colon, represented by principal component analyses (PCA; genus-level)

The amount of variance is depicted by the percentages on each axis. *Indicates significant differences at sow × offspring treatment level (P≤0.05); φ indicates sow treatment effect (P≤0.05).

Data from 36 pigs: Sow treatment level control n=18; FMT procedure (FMTP) n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12.

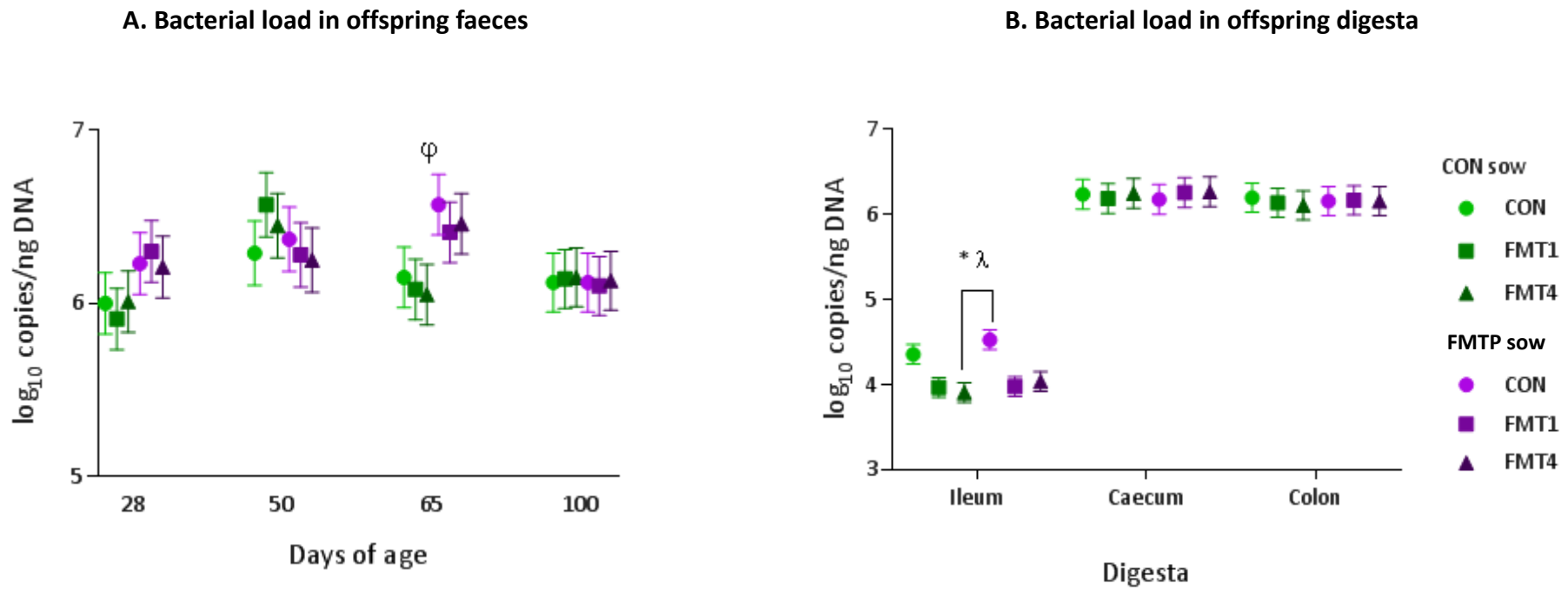


Figure S4.5. Effect of faecal microbiota transplantation (FMT) on faecal bacterial load (log_{10} copies 16S rRNA gene/ng DNA) in offspring A. faeces across all time points and B. intestinal digesta

Data from 36 pigs: Sow treatment level control n=18; FMT procedure (FMTP) n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12.

*Indicates significant differences at sow × offspring treatment level (P≤0.05); φ indicates sow treatment effect (P≤0.05); λ indicates offspring treatment effect (P≤0.05).

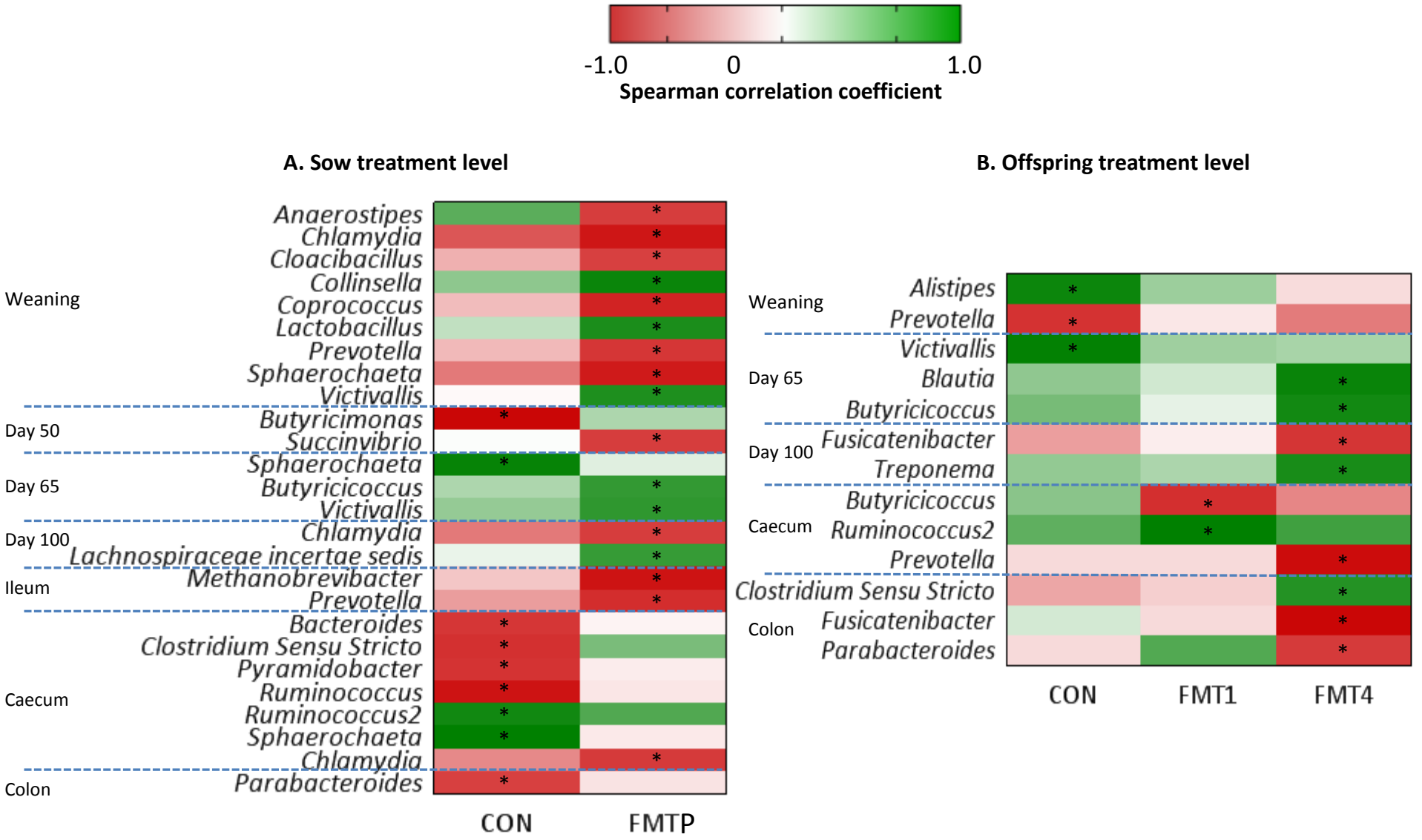
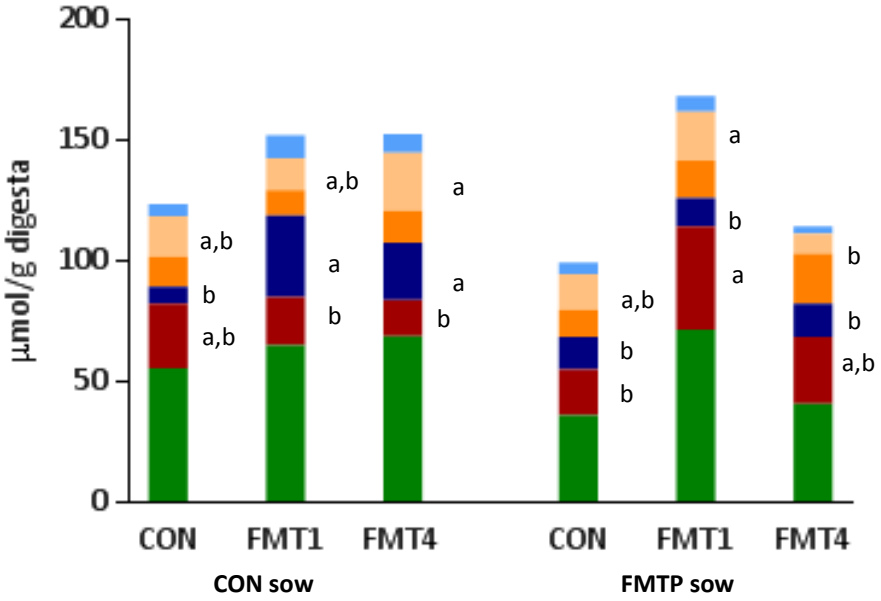

-1.0
0
1.0
Spearman correlation coefficient
A. Sow treatment level
Weaning
Day 50
Day 65
Day 100
Ileum
Caecum
Colon
Anaerostipes
Chlamydia
Cloacibacillus
Collinsella
Coprococcus
Lactobacillus
Prevotella
Sphaerochaeta
Victivallis
Butyricimonas
Succinvibrio
Sphaerochaeta
Butyricicoccus
Victivallis
Chlamydia
Lachnospiraceae incertae sedis
Methanobrevibacter
Prevotella
Bacteroides
Clostridium Sensu Stricto
Pyramidobacter
Ruminococcus
Ruminococcus2
Sphaerochaeta
Chlamydia
Parabacteroides
CON
FMTP
B. Offspring treatment level
Weaning
Day 65
Day 100
Caecum
Colon
Alistipes
Prevotella
Victivallis
Blautia
Butyricicoccus
Fusicatenibacter
Treponema
Butyricicoccus
Ruminococcus2
Prevotella
Clostridium Sensu Stricto
Fusicatenibacter
Parabacteroides
CON
FMT1
FMT4

Figure S4.6. Heat map showing Spearman correlations between bacterial genera and offspring body weight at day 155 of age due to faecal microbiota transplantation (FMT) in A. sows and B. offspring

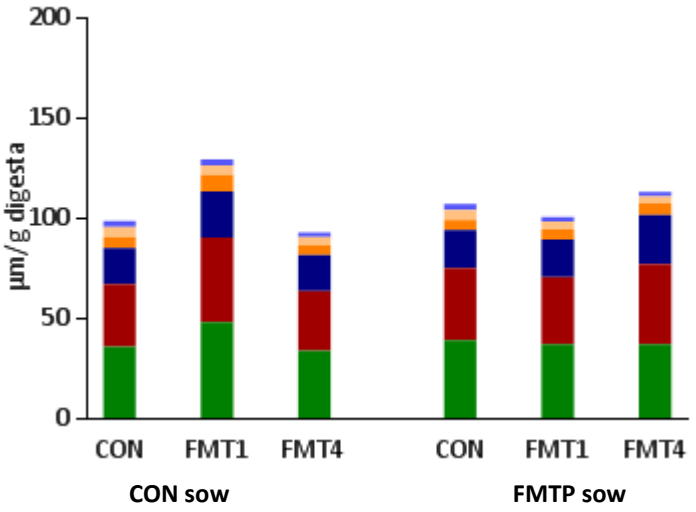
Data from 36 pigs: Sow treatment level control n=18; FMT procedure (FMTP) n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12.

*Denotes significant correlation within each treatment ($P \leq 0.05$).

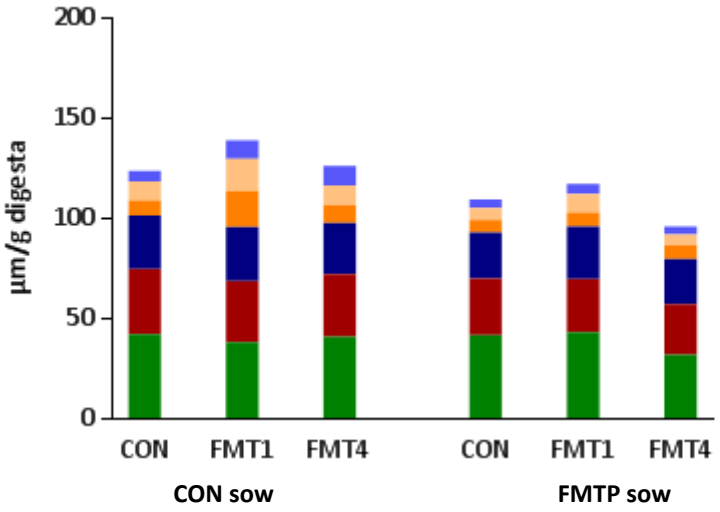
A. Ileal digesta



B. Caecal digesta



C. Colon digesta



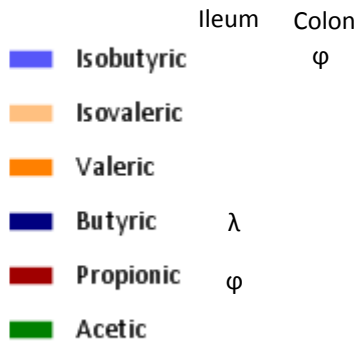


Figure S4.7. Effect of faecal microbiota transplantation (FMT) on volatile fatty acid concentrations in the A. ileum, B. caecum and C. colon of offspring at ~155 days of age

Data from 36 pigs: Sow treatment level control n=18; FMT procedure (FMTP) n=18; Offspring treatment level control n=12; FMT1 n=12; FMT4 n=12.

φ indicates difference at sow treatment level, λ indicates difference at offspring treatment level. ^{a,b,c} Within each row, values that do not share a common superscript are significantly different due to a sow × offspring treatment interaction ($P \leq 0.05$).

5. Seeking to improve feed efficiency in pigs through microbial modulation: faecal microbiota transplantation in sows and/or dietary supplementation of offspring with inulin

UM. McCormack, T. Curião, T. Wilkinson, BU. Metzler-Zebeli, H. Reyer, F. Crispie, PD. Cotter, CJ. Creevey, GE. Gardiner, PG. Lawlor. 2017. Improving feed efficiency through microbial modulation: faecal microbiota transplantation in sows and inulin feeding in piglets.

(Intended for submission to *Applied and Environmental Microbiology* September 2017)

5.1. Abstract

As previous studies have demonstrated a link between the intestinal microbiome and feed efficiency (FE), manipulating the microbiota may be a useful way to improve porcine FE. Faecal microbiota transplantation (FMT), using faecal extracts from highly feed efficient pigs, was performed in pregnant sows (n=11), with a control group (n=11) receiving no FMT. Subsequent offspring were weaned at ~28 d and allocated, within sow treatment, to either control group (n=67; no dietary supplements) or dietary inulin supplementation group (n=65) for six weeks. Microbiota transplantation in sows, alone or in combination with inulin supplementation of offspring, reduced offspring body weight by 8.1 – 10.6 Kg at slaughter (~140 days of age), but was associated with better FE, while inulin alone had no effects on growth or FE. The FMT alone, or the combined strategy increased bacterial diversity, and resulted in higher abundances of bacterial taxa considered beneficial e.g. *Bifidobacterium*, and *Butyricimonas*, and lower *Faecalibacterium* occurred in offspring faeces due to both treatments. There was a reduction in potentially pathogenic bacteria (*Chlamydia* and *Treponema*) in the ileum also. Both interventions resulted in lower caecal butyric acid concentrations, and maternal FMT led to a greater number of jejunal goblet cells in offspring. Inulin supplementation alone increased mean platelet volume, while reducing ileal propionic acid concentrations, granulocyte counts, and serum urea. Furthermore, duodenal genes linked to glucose and volatile fatty acid homeostasis was up-regulated in inulin-supplemented pigs. Overall, FMT in pregnant sows with/without dietary inulin supplementation modulated offspring intestinal microbiota (mostly low relative abundance taxa) and associated physiological parameters, and had a detrimental effect on growth, although FE was improved.

5.2. Importance

Intestinal health is key in terms of driving production performance in pigs, and the resident microbiota is critical for optimal gut health and metabolic functioning. As previous research suggests a link between the gut microbiota and FE, modulation of the intestinal microbiome may offer an effective means of improving FE in pigs. The FMT in gestating sows, in combination with post-weaning dietary inulin supplementation in offspring may be a novel way to achieve this. This combined approach (or maternal FMT alone) improved FE, and resulted in a number of differences in microbial composition and potential function, and other physiological parameters, which may help to explain the enhanced FE. However, the detrimental effects on lifetime growth will limit the practical application of these strategies in pig production, at least regimes employed in the present study. Nonetheless, additional research to identify specific prebiotics, or other dietary supplements that can be used to promote/maintain the microbiota implanted as a result of FMT to FMT, may be worthwhile in terms of optimising FE in pigs.

5.3. Introduction

Feed efficiency (FE) is of major importance in pig production, as feed accounts for the majority cost associated with producing pigs (Teagasc, 2016). Previous work from Chapter 2 and 3, and from others (Tan et al., 2017, Yang et al., 2017), has demonstrated an association between the intestinal microbiota and FE/ residual feed intake (RFI; metric for FE) in pigs. It may therefore be possible to improve FE through manipulation of the intestinal microbiota. This could potentially be achieved via faecal microbiota transplantation (FMT) and/or dietary supplementation with feed additives.

To date, the use of FMT in pigs has mainly been limited to the establishment of human microbiota in pigs in order to develop a model for humans (Pang, 2007, Zhang, 2013). However, pig microbiota have also been transferred to rodents (Hirayama, 1999) and to other pigs, although, to our knowledge, only three studies to date have performed the latter; one by Martin et al. (conference abstract) (Martin et al., 2015), one by (Xiao et al., 2017), and the other as detailed in Chapter 4. The study from Chapter 4, which used an inoculum derived from faecal extracts collected from highly feed efficient pigs with a view to improving FE (from Chapter 3) found that FMT in pregnant sows and/or their offspring impacted lifetime growth of offspring, as pigs were ~4-8 Kg lighter at slaughter. Intestinal microbiota composition and potential functionality, along with physiological parameters, were affected by FMT, and overall the results indicated that FMT may not be a suitable approach to optimise FE in pigs. Although some potentially beneficial FMT-associated modulation of the sow intestinal microbiota occurred, these effects were not transferred to/maintained in the offspring. Therefore, it is possible that dietary supplementation of the offspring with a prebiotic, may support the transplanted microbiota, potentially leading to a more positive outcome as regards FE.

Prebiotics are "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confers benefits upon host well-being

and health" (Roberfroid, 2007). Inulin is a dietary fibre derived mainly from chicory, and comprised of different chain lengths (2-65 monomers), which is not digestible by the host (Roberfroid, 2007). It has proved effective as a prebiotic in humans, but in pigs, results have been contradictory (Metzler-Zebeli et al., 2017b). However, a number of studies have found beneficial effects of supplementing inulin to pigs, both in terms of improving growth performance and modulating the intestinal microbiota (Grela et al., 2014, Patterson et al., 2010, Loh et al., 2006). In particular, supplementing weaner diets with inulin may be a useful way to counteract the susceptibility associated with the stress of weaning, and a number of studies have demonstrated beneficial modulation of the intestinal microbiota and improved growth, gut health, and FE, when inulin is fed to weaner pigs (Konstantinov et al., 2004, Awad et al., 2013, Patterson et al., 2010). However, as outlined above, conflicting results have been obtained. For example, Grela *et al.* found that dietary inulin addition improved weight gain, reduced feed intake and improved FE in pigs between 10 and 84 days of age (Grela et al., 2014), whereas a study conducted by Frantz *et al.* in 2003 found that inulin addition resulted in a deterioration in FE (Frantz et al., 2003). Inulin is fermented by enzymes produced from different bacterial members, resulting in increased production of volatile fatty acid (VFA) concentrations, mainly butyrate, and can improve host health and increase the absorption of minerals (Kelly, 2008). The addition of inulin to the diet of pigs (at various stages throughout their productive life) has been shown to increase bacterial populations considered beneficial, while reducing potentially pathogenic bacteria in the GIT (Kozłowska et al., 2016). However, reports on the microbiota-modulating ability of inulin are inconsistent and a recent meta-analysis showed that although strong negative relationships were found between dietary inulin and colonic enterobacteria throughout all production phases, the same was true for faecal lactobacilli (Metzler-Zebeli et al., 2017b).

Our hypothesis was that promoting the proliferation and persistence of an optimum microbial profile for improved FE early in life would improve lifetime FE in pigs. Our objective was to determine if FMT, using faecal extracts from highly feed efficient pigs, to pregnant sows and/or dietary supplementation of inulin to offspring post-weaning, would improve FE in offspring, and to determine if inulin supplementation would promote/maintain any beneficial bacteria potentially transferred to offspring as a result of maternal FMT.

5.4. Materials and methods

5.4.1. Ethical approval

The pig study was approved by the animal ethics committees of Teagasc (TAEC9/2013) and Waterford Institute of Technology (13/CLS/02) and performed according to European Union regulations outlining minimum standards for the protection of pigs (91/630/EEC) and concerning the protection of animals kept for farming purposes (98/58/EC). An experimental license (number AE1932/P032) was obtained from the Irish Health Products Regulatory Authority.

5.4.2. Animal management, recording and sampling

Faeces were collected from four highly feed efficient finisher pigs, anaerobically processed and the resultant faecal extracts prepared for use as FMT inoculum as described in Chapter 4. The same 22 sows in Chapter 4 were used here; on day 60 of gestation, sows were assigned to one of two treatment groups: 1) Control (n=11, CON), and 2) antibiotic treatment, purgative and FMT on days 70 and 100 of gestation (n=11, FMTP). The procedure for FMT was as described in Chapter 4. Briefly, on day 61 of gestation, FMTP sows received a 7-day course of an antibiotic cocktail with a broad antibacterial spectrum [Amoxicillin Trihydrate (Amoxinsol®, Vetoquinol UK Ltd., Buckingham, UK), 10 mg/Kg/day lincomycin-spectinomycin (Linco-Spectin® 100, Pfizer, Cork, Ireland) and 100,000 IU/Kg/day of colistin (Coliscour®, Ceva Sante Animale, Libourne, France)], followed by two doses of a purgative (sodium picosulfate, magnesium oxide and citric acid; Picolax powder, Ferring Ltd., Dublin, Ireland) to clear the GIT of resident microbiota, and a fasting period of 36 h. On days 70 and 100 of gestation, FMTP sows received the FMT (200 mL, which delivered a dose of ~2.6 × 10^{11} CFU) via gastric intubation along with a proton-pump inhibitor (Omeprazole;

Romep, Rowex Ltd., Cork, Ireland) to prevent inhibition of the bacteria in the inoculum by the acidity of the stomach.

A schematic depicting sow and offspring treatments and details of sampling is shown in Figure S5.1. At farrowing, the number of pigs born alive, stillbirths and mummies were recorded, as well as individual piglet birth weights and gender. All viable piglets were tagged for identification purposes, and litters remained intact as much as possible between farrowing and weaning. A commercial non-medicated starter diet (Table S5.1) was creep-fed between day 12 and weaning at ~day 28 of age.

At weaning, 132 pigs were selected across all litters, blocked by sow treatment, piglet gender and body weight, before being randomly assigned to single-gender pens, with 8-12 pigs per pen. Within sow treatment, pens of pigs were randomly assigned to: 1) control (6 pens; n=67 pigs; CON) 2) inulin for the first six weeks post-weaning (pw) (6 pens; n=65 pigs, INU). This resulted in a total of 4 treatments: control sow and control offspring (CON/CON), control sow and inulin-supplemented offspring (CON/ INU), FMTP-treated sow and control offspring (FMTP/CON), and FMT-treated sows and inulin-supplemented offspring (FMTP/ INU). Once weaned, piglets in both CON and INU groups were provided with the same sequence of diets (Table S5.1; starter for 1 week, followed by link for 2 weeks, followed by weaner for 3 weeks, followed by finisher to slaughter at ~140 days of age) except that for the INU group the starter and link diets contained 2% inulin (Orafti Synergy 1, 50:50 chain length, Beneo Animal Nutrition, Belgium) and the weaner diet contained 3% inulin. Pigs were provided with *ad-libitum* access to feed using the Feed Intake Recording Equipment (FIRE) feeding system (Schauer Agrotronic, Wels, Austria). The first week pw was regarded as a training period for the piglets, so feed intake for this period was not included in data analysis.

From weaning to ~78 days of age, pigs were housed in 12 fully slatted concrete (80 mm solid width, 18 mm slots) pens (2.4 m × 2.0 m). A canopy (2.4 m × 1.2 m) with 2 heat lamps was placed at the back of each pen to create a micro-climate and a suitable lying area was created using a solid rubber mat (2.4 m × 1.2 m) under the canopy. From ~78 days of age, the size of each pen was increased to 4.8 m × 2.4 m and the canopy and rubber mat were removed.

Body weight was recorded weekly and feed disappearance daily between ~35 and ~140 days of age from which performance indicators [Average Daily Feed Intake (ADFI), Average Daily Gain (ADG), Feed Conversion Efficiency (FCE)] were determined, and used to calculate RFI.

Any pigs treated with antibiotics were removed from the study. A total of 11 pigs were removed from the study due to health issues i.e. CON/CON (n=1): rectal prolapse; CON/INU (n=3): shoulder injury (n=1), navel rupture (n=2); FMTP/CON (n=4): lameness (n=1), navel rupture (n=3); FMTP/INU (n=3): lameness (n=1), navel rupture (n=2).

At ~140 days of age, all pigs were slaughtered by CO_2 stunning followed by exsanguination. Following evisceration, hot carcass weight was recorded, and multiplied by 0.98 to obtain cold carcass weight. Kill-out percentage was calculated as [(carcass weight/body weight at slaughter) × 100] and back-fat and muscle depth were measured at 6 cm from the edge of the split back at the third and fourth last ribs using a Hennessy Grading probe (Hennessy and Chong, Auckland, New Zealand). Lean meat yield was estimated according to the following formula: Lean meat yield = 60.30 – 0.847 X1 + 0.147 X2 [where X1= back-fat depth (mm) and X2= muscle depth (mm)].

Faecal sampling was conducted by rectal stimulation at 28 (weaning), 50, 65, 100 and 130 days of age on a subsample of 32 pigs (n=16 per sow treatment and n=16

per offspring treatment). Furthermore, digesta from the ileum, caecum and the colon were collected at slaughter from the same 32 pigs, as described in Chapters 2 and 4. All samples were snap frozen in liquid nitrogen and stored at -80 °C until microbiota and VFA analyses were conducted. Additionally, tissue from the duodenum, jejunum and ileum were collected from the 32 selected pigs for histological analysis and duodenal tissue scrapings were taken for both brush border enzyme and gene expression analyses, as previously described in Chapter 4.

5.4.3. DNA extraction, 16S rRNA gene sequencing and data analysis

Total DNA was extracted from faecal, ileal, caecal and colonic samples using the QIAamp DNA stool minikit (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions, apart from adding a beat beating step, and increasing the lysis temperature to 95°C to increase DNA yield (Buzoianu et al., 2012a).

The V3-V4 region of the 16S rRNA gene (~ 460 bp) was sequenced (2×250 bp paired end reads) on an Illumina MiSeq platform following the standard protocol with alterations, as in Chapter 2. Sequence reads were checked for quality using FastQC and trimmed to 240 bp in length at the end of the sequence using Trimommatic version 0.36 (Bolger et al., 2014) with adapters removed (Illumina CLIP software). Both reads were merged using Flash (Magoc and Salzberg, 2011) and quality checks were performed to guarantee maximum read coverage. Reads were then clustered into operational taxonomical units (OTUs) using a 97% sequence identity threshold. Chimeras were removed and reads were aligned to the CD-HIT specific database (version 111) and then Ribosomal Database Project classifier (RDP) was used for taxonomy assignments (Wang et al., 2007) applying a cut-off of 80% for unclassified taxa. Samples with less than 1,000 total reads were excluded from the analyses. The OTU data were scaled to the minimum number of total reads for each sample type and filtered to remove OTUs

present at <100 reads. Alpha-diversity indices [Chao1 (OTU richness), and Shannon and Simpson (OTU richness and abundance)] and beta-dispersion estimates were calculated using the Adonis2 and beta permutation functions of the Vegan package in R, each with 999 permutations. Principal component analysis (PCA) plots were generated with the bioconductor package deseq2 (Love et al., 2014) and ggplot in R.

5.4.4. Prediction of microbial function

The functionality of the microbiota for each sample based on 16S rRNA data and the 13_5 version of the Greengenes database for taxonomy and OTU assignments was predicted *in silico* using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved Species (PICRUSt) software (Langille et al., 2013). Prediction of functions was inferred based on Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations. Pathways not related with bacteria, not relevant to porcine studies and for which the relative abundance was <0.001% in samples were dismissed.

5.4.5. Volatile fatty acid concentration and pH

Concentrations of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids were measured in the ileal, caecal and colonic digesta according to Chapter 2. Briefly, ~8 g of sample was weighed and pH-recorded, diluted with trichloroacetic acid (x 2.5 times sample weight), and centrifuged (1,800 × *g* at 4 °C for 10 min). Subsequent supernatants were mixed with equal volumes of internal standard (i.e. 1.5 mL) and filtered into vials, and stored at -80 °C until analysis by gas chromatography (Agilent 5890 gas chromatograph) using hydrogen and helium as carrier gases, at 30 and 50 psi respectively, and temperatures of 80 °C (oven), 280 °C (detector), and 250 °C (injector).

5.4.6. Intestinal histology, disaccharidase activity and gene expression analysis

Intestinal tissue from the duodenum, jejunum and ileum (~3 cm) collected at slaughter was rinsed in PBS and placed in No-Tox fixative (Scientific Device Lab, Des Plaines, IL, USA) and placed on a shaker for 48 h. Samples were and stored at room temperature in the fixative until processing, which was performed as detailed in Chapter 4. Ten villi were examined per sample slide for villus height and width, crypt depth and goblet cell number using a light microscope at 400X magnification.

Duodenal mucosal scrapings were collected over a length of 10 cm for the analysis of disaccharidase activity and relative gene expression. Preparation of duodenal homogenates (20%, w/v) and mucosal enzyme activity measurements were performed as previously described (Metzler-Zebeli et al., 2017a). Target genes included intestinal alkaline phosphatase (*IAP*), facilitated glucose transporter member 2 (*GLUT2*), glucose-dependent insulinotropic peptide (*GIP*), glucagon-like peptide-1 (*GLP1*), monocarboxylate transporter 1 (*MCT1*) and sodium-coupled monocarboxylate transporter (*SMCT*), sodium/glucose cotransporter member 1 (*SGLT1*), tight junction proteins [occludin (*OCLN*) and zonula occludens 1 (*ZO1*)], and toll-like receptors (*TLR2* and *TLR4*). Total RNA was isolated from 20 mg duodenal mucosal scrapings using mechanical homogenization and the RNeasy Mini Kit (Qiagen, Hilden, Germany). Samples were homogenized using the FastPrep-24 instrument (MP Biomedicals, Santa Ana, CA, USA). After isolation, genomic DNA was removed by treating samples with the Turbo DNA kit (Life Technologies Limited, Vienna, Austria). The RNA was quantified using the Qubit HS RNA Assay kit on the Qubit 2.0 Fluorometer (Life Technologies Limited, Vienna, Austria) and the quality of extracted RNA evaluated with the Agilent Bioanalyzer 2100 (Agilent RNA 6000 Nano Assay, Agilent Technologies, Waghaeusel-Wiesental, Germany). Complementary DNA was

synthesized from 2 µg RNA using the High Capacity cDNA RT kit (Life Technologies Limited) and 1 µL of RNase inhibitor (Biozym, Hessisch Oldendorf, Germany) was added to each reaction. Primers used for qPCR are listed in Table S5.2.

The primers were verified with PrimerBLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/) and tested for efficiencies and specificity using melting curve analysis (Table S5.2). Amplifications were performed on a real-time PCR Mx3000P (Agilent Technologies) thermocycler using the following conditions: 95°C for 5 min, followed by 95°C for 10 s, 60°C for 30 s and 72°C for 30 s for 40 cycles, followed by the generation of melting curves. Negative controls and reverse transcription controls (RT minus) were included in order to control for residual DNA contamination. Each 20 µL reaction consisted of 50 ng cDNA, 10 µL Fast Plus Eva Green master mix with low ROX (Biotium, Hayward, CA, USA), 100 nM each of forward and reverse primers and 10 µL DEPC-treated water in a 96 well plate (VWR, Vienna, Austria). All reactions were performed in duplicate as previously described (Metzler-Zebeli et al., 2017a).

5.4.7. Haematology and blood biochemistry analyses

Blood was collected during exsanguination at slaughter for haematology and biochemistry analyses from the 32 selected pigs. For haematological analysis, blood was collected in vacuette tubes (Labstock, Dublin, Ireland) containing EDTA to prevent clotting, and analyzed within 4 h using a Beckman Coulter Ac T Diff analyzer (Beckman Coulter Ltd., High Wycombe, UK).

For biochemical analysis, blood was collected in vacuette tubes (Labstock, Dublin, Ireland) and allowed to clot at room temperature, followed by centrifugation at 1,500 × *g* for 10 min. The serum was then collected and stored at -80 °C for subsequent analysis. Concentrations of total protein, blood urea nitrogen, glucose, triglycerides,

cholesterol, creatinine and creatine kinase were measured using an ABS Pentra 400 clinical chemistry analyser (Horiba, ABX, North Hampton, UK). The analyzer was calibrated according to the manufacturer's instructions and every fifth sample was analysed in duplicate to determine analyser accuracy.

5.4.8. Statistical analysis

Growth performance parameters recorded throughout the study were analysed for repeated measures using PROC MIXED in SAS 9.3 (SAS, 2011), with gender, boar, and treatment (sow/offspring) used as fixed effects. Pig nested within pen was used as a random effect to account for variability regarding pen assignment. The RFI was calculated between day 35 and ~140 days of age (at slaughter) as the residuals from a least squares regression model of ADFI on ADG, metabolic live-weight, gender and all relevant two-way interactions, as well as the effects of back-fat and muscle-depth which were recorded at slaughter.

Intestinal histology, gene expression, brush border enzymatic activity, and blood parameters (haematology and serum biochemistry) were also analysed using the MIXED procedure in SAS 9.3, where similar models as for growth performance used. A generalised linear mixed model using PROC GLIMMIX in SAS 9.3 was used to analyse VFA concentrations, which were deemed "not-normal", following log transformation.

Microbial composition and predicted functionality data were analysed using generalised linear mixed model equation methods in PROC GLIMMIX of SAS 9.3. A gamma distribution was assumed for all data. Models for offspring bacterial relative abundance for the faecal time points and digesta included sow treatment, offspring treatment, faecal sampling time point and their interactions as fixed effects. Additionally, a random intercept for each faecal time point was included to account for

the repeated measurements. Microbial composition and predicted functionality for which relative abundance was present at <0.001% were dismissed. In all models, data were back-transformed to the original distribution using the *ilink* option in PROC GLIMMIX. For all analyses, statistical significance was set at $P<0.05$. Heatmaps used to depict relative abundance differences between treatments (for microbial composition and predicted functionality) were generated in GraphPad prism 7.

5.5. Results

5.5.1. Impact of FMT in sows and/or inulin inclusion in offspring diets on offspring growth

The effect of FMTP to sows and inulin inclusion in offspring diets on offspring growth is shown in Table 5.1. An interaction was observed for offspring body weight at 100 days of age, where FMTP/CON had lighter body weight compared to CON/CON and CON/INU ($P<0.05$), and offspring from FMTP sows were lighter compared to their control counterparts also ($P<0.05$). At ~140 days of age (slaughter), FMTP/CON and FMTP/INU offspring were 10.6 and 7.1 Kg lighter respectively ($P<0.05$), than control and inulin supplemented offspring from control sows ($P<0.05$). Due to FMTP in sows, offspring were also lighter at slaughter ($P<0.05$). Consequently, the cold carcass weights of offspring from FMTP sows were 8.9 and 5.1 Kg lighter ($P<0.05$) than those of offspring from control sows when offspring treatment was control and inulin, respectively ($P<0.05$). There was also an interaction for muscle depth in the carcass, where FMTP/INU had a greater muscle depth compared to CON/INU offspring ($P<0.05$). No treatment differences were observed for ADFI, ADG, FCE or other carcass traits measured in offspring.

There was an interaction between sow treatment and offspring treatment ($P<0.05$) for RFI. Offspring from FMTP sows (FMTP/CON and FMTP/INU) had a lower RFI value (better FE) compared to CON/INU offspring ($P<0.05$). This was reflected at sow treatment level, where pigs from FMTP sows had a lower RFI than those from CON sows ($P<0.05$). Inulin alone did not influence offspring RFI ($P>0.05$).

5.5.2. Influence of FMTP in sows and/or inulin inclusion in offspring diets on offspring intestinal microbial diversity

Significant treatment effects for microbial diversity are shown in Figure 5.1. At 100 days of age, FMTP/INU pigs had enhanced species richness (Chao1 index; number of rare OTUs), compared to offspring from CON sows ($P<0.05$; Figure 5.1A). At 130 days of age, all treatments had higher Shannon diversity (species evenness) compared to CON/CON ($P<0.05$; Figure 5.1B) and irrespective of offspring treatment, offspring from FMTP sows had a higher Shannon diversity (4.2) than offspring from CON sows (3.8; $P<0.05$; data not shown). However, lower Simpson diversity (species evenness) was observed in the ileum of inulin-supplemented offspring (0.66) compared to control offspring (0.71; $P<0.05$; data not shown).

Microbial diversity (ß-diversity) was also measured in all faecal and intestinal samples, and is depicted using principal component analysis (PCA) plots (Figure S5.2). Throughout the lifetime, there was an influence of sow treatment on offspring microbial diversity, with offspring from FMTP sows clustering away from offspring born to CON sows in the faeces at weaning ($P<0.05$), and tending to cluster on days 50 ($P=0.06$), 65 ($P=0.07$) and 100 ($P=0.08$) of age. On day 65, inulin treatment also led to a clustering effect ($P<0.05$). Just prior to slaughter, at ~130 days of age, an influence of sow treatment was observed again ($P<0.05$). Although there were no significant differences in the ileum, CON/CON and FMT/CON samples clustered separately in the caecum ($P<0.05$), and in the colon, CON/INU and FMTP/CON clustered away from CON/CON offspring ($P<0.05$).

5.5.3. Effect of FMTP in sows and/or inulin supplementation of offspring on offspring intestinal microbial composition

Microbial composition, at the phylum and genus level, was investigated in offspring faeces throughout their lifetime and in the intestinal digesta collected at slaughter. Composition at phylum level for faeces and digesta samples are shown in Figure 5.2. Twelve phyla were detected in the faeces at weaning, 8 at day 50 of age, 6 at day 65 of age, 15 at day 100 of age and 14 at day 130 of age, with 8 detected in the ileum, and 7 in both the caecum and colon, respectively.

A total of 9 phyla and 37 genera differed between treatments, and these ranged in relative abundance from 0.04-18.6% and 0.02-17.3%, respectively, but were mainly present at low relative abundance (Figure 5.3A, Table S5.3). Five phyla and 19 genera differed due to a sow × offspring treatment interaction, 8 phyla and 31 genera due to sow treatment (Figure 5.3A), and 4 phyla and 20 genera due to offspring treatment (Figure 5.3B).

At weaning, *Lentisphaerae*, Synergistetes and *Actinobacteria* were higher in relative abundance in offspring due to FMTP in sows (Figure 5.3A; $P<0.05$), but *Lentisphaerae* was lower in the inulin-supplemented group, although inulin was only added to the diet at weaning (Figure 5.3 B; $P<0.05$). Interestingly, *Proteobacteria* was impacted throughout the lifetime of the pig, mainly due to sow treatment. In the faeces at day 50, FMTP in sows resulted in a higher relative abundance of Proteobacteria in offspring (Figure 5.3A and Table S5.3; $P<0.05$) but this phylum was lower in abundance due to inulin supplementation (Figure 5.3B; $P<0.05$). This FMTP effect was also observed in the faeces collected on day 100 (Figure 5.3B; $P<0.05$), and in the caecum as well (Figure 5.3A and Table S5.3; $P<0.05$). On day 100, *Fusobacteria* and was higher, whereas *Fibrobacteres* was lower in relative abundance in offspring from FMTP in sows (Figure 5.3A; $P<0.05$) and30 days later, *Fibrobacteres* remained at a

lower abundance due to FMTP (Figure 5.3A and Table S5.3; P<0.05), but was higher in relative abundance due to inulin supplementation (Figure 5.3B; P<0.05). Also on day 130 of age, *Actinobacteria* was higher in relative abundance, and in the ileum, *Spirochaetes* was lower, in offspring due to FMTP in sows (Figure 5.3A and Table S5.3; P<0.05). Furthermore, *Chlamydiae* was reduced in all groups compared to CON/CON offspring (Table S5.3; P<0.05), and was also reduced due to dietary inulin supplementation (Figure 5.3B; P<0.05). *Tenericutes* was present at a higher relative abundance in the caecum of offspring born to FMTP sows, compared to their control counterparts (Figure 5.3A; P<0.05).

Most of the treatment differences at genus level occurred in the faeces at weaning, or just prior to slaughter, at day 130 of age, and in the ileal digesta at slaughter (Figure 5.3C and D; P<0.05). Apart from *Spirochaeta* (day 130 of age) and *Treponema* (ileal digesta), all the differences observed were for genera present at <5%relative abundance.

Throughout the lifetime of the pigs, a number of bacterial genera were impacted at more than one faecal time point as well as in the digesta collected at slaughter, with a strong effect of sow treatment seen over time. However, the results did not always follow the same trend across different time points. For example, due to sow FMTP *Butyricicoccus* was present at a higher relative abundance in the offspring at weaning, but was lower in the caecum, and *Treponema* was also higher at weaning but lower in abundance in the ileum of offspring due to sow FMT (Fig 3C; P<0.05).

Mainly due to FMTP in sows, *Bifidobacterium* was present at a higher at weaning and in the faeces collected 30-60 days post-inulin removal from the diet (Figure 5.3C and D, and Table S5.3; P<0.05). Furthermore, *Eubacterium* was more abundant in the faeces collected at weaning and on day 100 of age in the offspring born

to FMTP sows, compared to their control counterparts (P<0.05). Although *Blautia* was present at a lower abundance in the faeces of inulin-supplemented offspring at weaning (albeit inulin supplementation only commenced at weaning), compared to control offspring (Figure 5.3D), it was higher in abundance in the faeces collected at 130 days of age (60 days post-inulin removal) in FMTP/INU offspring compared to FMTP/CON offspring (Table S5.3; P<0.05), and was higher in abundance due to inulin supplementation also (Figure 5.3; P<0.05).

At weaning, due to the combination of FMTP/INU, *Faecalibacterium* was lower, whereas *Butyricimonas* was higher in relative abundance compared to all other groups (Table S5.3; P<0.05), and due to FMTP in sows, *Faecalibacterium* was lower but *Butyricimonas* was higher compared to offspring from CON sows (Figure 5.3C; P<0.05). Furthermore, *Dorea* was lower in abundance in FMTP/CON offspring compared to inulin-supplemented offspring, regardless of sow treatment (Table S1, Fig. 3D; P<0.05), and was less abundant due to FMTP in sows also (Fig. 3C; P<0.05). *Terrisporobacter* was enhanced due to FMTP in sows, but was lower in abundance due to dietary supplementation of inulin (Figure 5.3C and D, and Table S5.3; P<0.05). Throughout the production period (days 28-130 of age), FMTP in sows resulted in a lower faecal relative abundance of another three bacterial genera, and a higher abundance of another two bacteria genera (Fig 3C; P<0.05). In the faeces collected throughout the growing period (days 50 and 100 of age), *Campylobacter* was also impacted due to both FMTP and inulin supplementation (Table S1, Fig. 3C and D; P<0.05). In the faeces collected on days 100 and 130 of age, *Acetanaerobacterium* and *Sutterella* were higher, whereas *Fibrobacter* was lower in relative abundance, mainly due to FMTP in sows (Figure 5.3C and D, Table S5.3: P<0.05). In the ileum, *Prevotella* was present at a higher relative abundance, whereas *Chlamydia* was lower in all groups compared to CON/CON (Figure 5.3C and D, Table S5.3; P<0.05).

Throughout the growing period, FMTP in sows resulted in lower abundance of four genera, and a higher abundance of four genera (Fig. 3C; $P<0.05$), and in the ileum, a further three bacterial genera were enhanced (Fig. 3C; $P<0.05$). Due to dietary inulin supplementation, four genera were also lower and one was higher in the faeces collected throughout the growing period (Fig 3D; $P<0.05$). In the caecum, *Bacteroides* was higher due to FMTP, but reduced due to inulin supplementation (Table S1, Fig 3C and D; $P<0.05$).

5.5.4. Effect of FMTP in sows and/or inulin supplementation of offspring on predicted functionality of the offspring intestinal microbiota

The functionality of the intestinal microbiota was predicted in all offspring faecal and digesta samples, and significant differences due to sow and offspring treatment are shown in Figure 5.4. A total of 20 predicted bacterial pathways in offspring were significantly impacted due to a sow × offspring treatment interaction (Table S5.3). As a result of FMTP in sows, 27 pathways were altered in the offspring, mostly related to lipid metabolism, carbohydrate metabolism and xenobiotics degradation and metabolism (Figure 5.4A). Due to dietary inulin supplementation in offspring (Figure 5.4B), 22 predicted pathways, mostly related to carbohydrate metabolism and glycan biosynthesis and metabolism were impacted. Overall, most of the effects were seen within the ileal microbiota.

In the faeces collected at weaning, predicted pathways related to biosynthesis of other secondary metabolites and carbohydrate metabolism were less and more abundant in offspring from FMTP sows, respectively (Figure 5.4A; $P<0.05$). No differences were observed in the faeces of 50-day old pigs, but at 65 days of age a pathway relating to metabolism of cofactors and vitamins was predicted to be higher due to offspring inulin supplementation (Figure 5.4B; $P<0.05$).

Notably, in the faeces collected at 130 days of age, pathways relating to nucleotide and terpenoid and polyketide metabolism were impacted due to FMTP in sows and/or dietary supplementation of inulin to offspring, compared to CON/CON offspring (Figure 5.4A and B; $P<0.05$). Also, inulin supplementation enhanced the predicted abundance of a fatty acid metabolism pathway (Figure 5.4B; $P<0.05$).

In the ileum, pathways related to lipid metabolism were predicted to be at a higher abundance in offspring from FMTP sows (Figure 5.4A; $P<0.05$), and/or in offspring supplemented with inulin (Figure 5.4B; $P<0.05$). A pathway involved in xenobiotic degradation and metabolism was predicted to be higher in offspring due to FMTP treatment in sows, but lower in abundance due to dietary supplementation of inulin to offspring, whereas the opposite occurred for a pathway related with metabolism of other amino acids (Figure 5.4A and B; $P<0.05$). A pathway related with metabolism of terpenoids and polyketides was also predicted to by more abundant in offspring, due to FMTP treatment in sows (Figure 5.4A; $P<0.05$), whereas a pathway involved in the metabolism of co-factors and vitamins was lower in abundance due to inulin supplementation of offspring (Figure 5.4B; $P<0.05$). Moreover, another five predicted pathways involved in lipid and carbohydrate metabolism and xenobiotics degradation and metabolism were higher in abundance, and a further five predicted pathways related with amino acid metabolism, biosynthesis of other secondary metabolites, glycan biosynthesis and metabolism and metabolism of co-factors and vitamins were predicted to be less abundant in offspring due to FMTP in sows (Figure 5.4A; $P<0.05$). Six other pathways involved in glycan biosynthesis and metabolism, biosynthesis of other secondary metabolites, and metabolism of other amino acids were predicted to be higher, and a further two pathways related with carbohydrate metabolism and biosynthesis of other secondary metabolites were lower in abundance due to inulin supplementation of offspring (Figure 5.4B; $P<0.05$).

In the caecum, a lower predicted abundance of a pathway involved in the metabolism of other amino acids was observed in offspring from FMTP sows (Figure 5.4A; $P<0.05$), but offspring supplemented with inulin had a higher predicted abundance (Figure 5.4B; $P<0.05$). In contrast, pathways related to carbohydrate metabolism were predicted to be higher in offspring from FMTP sows, although they were present at low relative abundance (Figure 5.4A; $P<0.05$), while inulin treatment resulted in a lower predicted abundance (Figure 5.4B; $P<0.05$). Three other predicted pathways related to carbohydrate and lipid metabolism, and xenobiotic biodegradation and metabolism were higher due to FMTP in sows, and another three (metabolism of terpenoids and polyketides, lipid metabolism, and metabolism of co-factors and vitamins) were predicted to be less abundant due to FMTP in sows (Figure 5.4A; $P<0.05$). In the caecum of offspring supplemented with inulin, a predicted pathway related to biosynthesis of other secondary metabolites was enriched, but pathways related to carbohydrate and energy metabolism were lower in abundance compared to control offspring (Figure 5.4B; $P<0.05$). In the colon, a pathway involved in lipid metabolism was predicted to be more abundant in offspring due to FMTP in sows (Figure 5.4A; $P<0.05$).

5.5.5. Effect of FMTP in sows and/or inulin supplementation of offspring on offspring intestinal volatile fatty acid concentrations

Volatile fatty acid concentrations were measured in digesta from the ileum, caecum and colon of the 32 selected offspring, and results are shown in Figure 5.5. No differences were observed between treatments for digesta pH, in any of the intestinal segments (data not shown). In the ileum, offspring from FMTP/INU had higher concentrations of acetic acid compared to the other groups, and CON/INU had lower

propionic acid concentrations compared to CON/CON offspring (P<0.05), and this VFA was also reduced in inulin-fed offspring (Figure 5A; P<0.05).

In the caecum, butyric acid concentrations were lower for FMTP/INU compared to all other groups, and for FMTP/CON compared to both offspring treatments from control sows (P<0.05). It was also lower due to FMT in sows and inulin intake in offspring (Figure 5.5B; P<0.05). Moreover, valeric acid was lower in FMTP/INU compared to all other groups, but CON/INU pigs had a higher concentration compared to control offspring, regardless of sow treatment (P<0.05) Due to FMTP in sows, offspring had a lower concentration present also (Figure 5.5B; P<0.05). However, isovaleric acid concentrations were higher in FMTP/CON, but lower in FMTP/INU, compared to all other groups (P<0.05), and was lower due to inulin also (Figure 5.5B; P<0.05).

In the colon, isobutyric acid concentrations were higher in FMTP/CON compared to all other groups (P<0.05), and higher due to FMTP in sows (Figure 5.5C; P<0.05).

5.5.6. Influence of FMTP in sows and/or inulin supplementation of offspring on offspring intestinal histology

Histological measures of the offspring small intestine (duodenum, jejunum, and ileum) are shown in Figure 5.6. In the duodenum, none of these differed between groups. However, FMT/CON had a higher number of jejunal goblet cells per µm villus height compared to CON/CON offspring (Figure 5.6B; P<0.05), and a higher number of goblet cells (per villus and per µm villus height) was also observed due to FMTP in sows (Figure 5.6B and C; P<0.05). The FMTP in sows resulted in shorter ileal villi and a smaller villus area compared to CON sows (Fig 5A; P<0. 05).

5.5.7. Influence of FMTP in sows and/or inulin supplementation of offspring on offspring brush border enzymes and gene expression in the duodenum

Disaccharidase activity in the duodenum of offspring at slaughter (~140 days old) is shown in Figure 5.7A. A sow × offspring treatment interaction was observed for maltase activity only, where CON/INU had a lower activity compared to CON/CON and FMTP/INU offspring, and the latter had a higher activity compared to FMTP/CON offspring ($P<0.05$). No differences at sow or offspring treatment level were observed ($P>0.05$).

Expression of 3 of the 11 genes measured in the duodenum were impacted as follows (Figure 5.7B): an up-regulation of *GIP* was observed in CON/INU compared to CON/CON offspring, and this was also observed in inulin-supplemented compared to control offspring ($P<0.05$). In addition, *GLP1* and *SMCT* were up-regulated in inulin-supplemented offspring compared to their control counterparts ($P<0.05$).

5.5.8. Influence of FMTP in sows and/or inulin supplementation of offspring on offspring blood parameters

The results of offspring haematological analysis at slaughter are shown in Table 5.2. White blood cells were lower in CON/INU compared to CON/CON offspring, and tended to be lower in inulin supplemented pigs also (26.6 vs. 23.8 $x10^3$ cells/μL; $P=0.09$). Both granulocyte percentage (64 vs. 54) and number (17 vs. 11) were lower in inulin-supplemented compared to control offspring ($P<0.05$), and platelet volume was higher (10.3 vs. 9.5; $P<0.05$). In addition, haemoglobin was higher in FMTP/INU vs. FMTP/CON offspring ($P<0.05$), and mean corpuscular haemoglobin percentage was lower in offspring from FMTP sows compared to their control counterparts (17.8 vs. 18.8; $P<0.05$).

Of all biochemical measures made in offspring at slaughter (Table S5.4), only cholesterol (P=0.07) and urea (P=0.06) concentrations tended to be lower. Cholesterol was lower in both offspring treatments from FMTP sows compared to CON/CON, whereas blood urea nitrogen was reduced due to inulin supplementation (11.1 vs. 16.3 mg/dL).

5.6. Discussion

Beneficial modulation of the intestinal microbiota may result in improved intestinal health and nutrient utilisation, and ultimately, improved growth and FE in pigs. Prebiotics, most notably inulin, have been studied in pigs in order to achieve this (Metzler-Zebeli et al., 2017b, Kozłowska et al., 2016, van der Aar et al., 2016, Grela et al., 2016). Microbiota transplantation may also be a useful approach, as it has been shown to transfer host physiological traits, such as leanness, obesity and gut characteristics, via 'reprogramming' of the intestinal microbiota (Diao et al., 2016, Yan et al., 2016, Ridaura et al., 2013, Ellekilde et al., 2014) However, the work in Chapter 4 showed a depression in offspring body weight at slaughter as a result of FMT in sows and/or offspring. Nonetheless, some beneficial modulation of the intestinal microbiota occurred in pregnant sows receiving FMT, and so here we tested the hypothesis that dietary supplementation of subsequent offspring with a prebiotic (inulin) would maintain and promote any beneficial bacterial transferred from the sows as a result of FMTP.

Results showed that pigs born to FMTP sows (irrespective of post-weaning treatment) were 8.9 Kg lighter at slaughter, but were more feed efficient, given their lower RFI value. Post-weaning dietary supplementation with inulin has previously resulted in increased weight gain and improved FE in pigs (Metzler-Zebeli et al., 2017b, Grela et al., 2014), although in some cases, results are contradictory (Frantz et al., 2003). In the present study, no improvements in weight gain, or indeed FE, were observed due to inulin inclusion in post-weaning diets alone.

However, FE was improved when inulin was supplemented to the diet of weaner pigs born to FMT-treated sows (albeit body weight was reduced), so it may have a role in promoting the proliferation of beneficial bacterial populations implanted in the GIT early in life as a result of modulation of the maternal microbiota. Interestingly, due to

the combined strategy, *Prevotella* was higher in abundance compared to the control offspring from control sows. Given that FE was improved in inulin-supplemented offspring from FMTP sows, this contradicts recent findings that associate this genus with poor FE (high RFI and FCR values) (Tan et al., 2017, Yang et al., 2017). Furthermore, *Prevotella* is a key genus previously associated with weight gain (Mach et al., 2015) and reduced adiposity in pigs (Yang et al., 2016). However, weight gain was not observed in the present study and, although adiposity was not directly measured, back fat and lean meat yield did not differ between treatments.

A higher abundance of bacteria deemed beneficial for host health was also found in offspring faeces due to either FMTP in sows (most pronounced) or offspring inulin supplementation. Most of these effects were observed at weaning; in fact for inulin treatment all were found at weaning which is meaningless as inulin supplementation only commenced then. However, for the maternal FMT treatment some of the effects were repeatedly found throughout the growing period. The taxa enriched included the lactic acid-producing bacteria, *Bifidobacterium*, *Streptococcus* and *Lactobacillus*, as well as butyric acid-producing bacteria, such as *Eubacterium*, *Coprococcus* and *Butyricimonas* (Sakamoto et al., 2009). The increased abundance of some of these microbes may be related to metabolic cross-feeding, with lactate produced by the lactic acid bacteria being utilised by these taxa and converted to butyric and propionic acids (Louis and Flint, 2017), although both of these acids were at lower concentrations due to FMTP and/or inulin. Interestingly, *Bifidobacterium* has been shown to improve growth performance and digestibility of dietary components in weaner pig diets (Yang et al., 2015, Zhao et al., 2015, Patterson et al., 2010) and is associated with the digestion of oligosaccharides from maternal milk (Flint et al., 2012). However, this genus was only enhanced in inulin-supplemented pigs at weaning (prior to commencement of treatment) and was, in fact, less abundant in this group at day 100.

Although FMT in sows and dietary inulin supplementation to offspring had a similar outcome in terms of effects on the relative abundance of certain bacterial taxa, as outlined above, contrasting effects were also observed for the phyla *Lentisphaerae*, *Proteobacteria* and *Fibrobacteres*. At the genus level, *Ruminococcus*2, found to play a role in degradation of resistant starch (Sun et al., 2015), was increased in offspring as a result of FMTP in sows, which is in agreement with previous findings in Chapter 4, that it was also enriched in the faeces of the sows themselves. However, offspring inulin supplementation alone reduced its abundance. This was also the case for *Bacteroides*, a genus known to be hemicellulolytic, although the opposite was true for the cellulolytic genus *Fibrobacter*(Flint et al., 2012, Martens et al., 2009), which was less abundant in offspring due to maternal FMT but more abundant due to offspring inulin supplementation. Interestingly, *Bacteroides* was previously found to be associated with low RFI in the faeces of 160 day old pigs from Chapter 2, and considering the higher abundance of *Bacteroides* in the caecum of offspring from FMTP sows, this may explain the improved FE observed in the present study. Additionally,

Interestingly, overall, treatment effects were more evident within the faecal microbiome of pigs at the end of the finishing period, i.e. at 100 and 130 days of age, even though inulin was removed from the diet 30-60 days prior to this and, and FMT was performed in the sows only, demonstrating that effects of both treatments persisted throughout the productive life of the pig.

Similar to the results obtained here for finisher pigs, a study conducted in piglets found that inulin did not affect pig weight gain, colon crypt depth, or villus height in either the jejunum or ileum (Kien et al., 2007). The maternal influence alone on offspring microbiota was evident throughout the current study, not only in terms of composition, but also potential function, especially in relation to pathways associated with carbohydrate and lipid metabolism. Purine metabolism, the bacterial pathway

predicted at the highest relative abundance in the current study, decreased due to a combination of FMTP in sows and inulin supplementation of offspring. This pathway has been negatively correlated with members of *Ruminococcaceae* (*Firmicutes*) in the early life of pigs (Merrifield et al., 2016) and some taxa within this family (e.g. *Butyricicoccus*, *Faecalibacterium*) were reduced in offspring in the present study both by maternal FMT and inulin supplementation. In agreement with the fact that inulin is a plant-storage glycan, the microbiota of inulin-supplemented offspring had an enhanced predicted abundance of glycan biosynthesis and metabolism pathways, and a lower abundance of other carbohydrate metabolism pathways, although a concomitant increase in VFA concentrations was not observed, in contrast with previous findings in humans (Koropatkin et al., 2012).

Although there was an increase in relative abundance of beneficial microbes due to sow and/or offspring, a reduction in key butyrate producers e.g. *Butyricicoccus* in the caecum, may have accounted for the concomitant reduction in butyric acid concentrations observed in the caecum of inulin-supplemented offspring born to FMT-treated sows. This is in contrast to previous findings that inulin increased butyric acid concentrations in the caecum (Grela et al., 2016). This microbial metabolite is important as it is the primary source of energy for colonocytes and has been shown to increase cell proliferation in different intestinal segments, including the caecum (Kien et al., 2007, Jiang et al., 2015).

Genes involved in glucose homeostasis, in particular the secretion of insulin, such as *GIP* and *GLP1* were more abundant in the duodenum of inulin-supplemented pigs. This is likely indicative of inulin fermentation in the upper GIT, or perhaps a compensatory mechanism for nutrient digestion in the small intestine, potentially leading to a better metabolic capability of pigs supplemented with inulin. Furthermore, a higher utilisation of protein/nitrogen by the microbiota (Halas et al., 2010) may have

occurred, as indicated by lower serum urea concentrations in inulin-fed offspring. Inulin has also been linked with possible lipid-modulatory effects in humans and piglets (Davidson et al., 1999, Grela et al., 2014), which is in accordance with the reduced serum cholesterol concentration found in the present study. Furthermore, the reduced cholesterol concentration observed may be due to the higher concentrations of acetic acid present, as acetic acid has been found to reduce the levels of serum cholesterol, when added to the diet of rats (Fushimi et al., 2007).

Inulin has been shown to modulate not only growth and FE but also immunological properties in pigs (Grela et al., 2014). Interestingly, white blood cell and granulocyte counts decreased due to FMT in sows and/or inulin supplementation of offspring, and the lower counts of these immune cells may be linked to the lower abundance of potential pathogens observed in these pigs (e.g. *Campylobacter*, *Chlamydia* in the faeces).This in turn may be due to the higher abundance of lactic acid bacteria in these animals, which are known to reduce pathogens in the GIT (Naidu et al., 1999). Moreover, offspring from FMTP sows may have an over-enhanced production of mucin in the small intestine, as more goblet cells were present in the jejunum and mucin is a physical barrier which prevents pathogenic bacteria from adhering to the epithelial lining (Kim et al., 2010). Previous work from Chapter 2 found that less feed efficient pigs had a higher number of ileal goblet cells, and in relation to the present study this may further explain the reduced body weight at slaughter observed in offspring born to FMTP-treated sows.

5.7. Conclusion

We provide evidence that strategies to modulate the intestinal microbiota of pigs (specifically maternal FMT alone or in combination with dietary inulin supplementation in offspring) have a huge impact on pig growth and FE throughout their productive lifetime, albeit detrimental in terms of body weight but beneficial as regards FE. These effects were accompanied by influences on both intestinal microbiota and predicted functionality in the offspring. Although dietary supplementation with inulin alone had a similar impact on the intestinal microbiota, effects were not as pronounced and improvements in offspring growth or FE were not observed. As regards effects on offspring intestinal microbiota, in particular, bacterial taxa considered beneficial such as *Bifidobacterium*, and *Butyricimonas*, albeit mainly present at low relative abundance, were increased mainly due to FMTP in sows. Some contrasting influences were also observed on bacterial members due to the combinative effect of FMTP in sows and/or offspring dietary inulin supplementation. Taken together, the haematological, biochemical and gene expression data suggest improved health in offspring from FMTP-sows, and/or those supplemented with inulin. Overall, the results from this study show that maternal FMT, either alone or in combination with post-weaning inulin supplementation, is not suitable for use in pig production (at least not the regime used in the present study) due to the detrimental impact on lifetime growth. Nontheless, additional research to identify specific prebiotics or other dietary supplements that can be used to promote/maintain the microbiota transferred as a result of maternal FMT, may be worthwhile in terms of optimising FE.

5.8. Tables and Figures

Table 5.1 Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on pig growth performance and carcass traits[1]

Sow treatment	**Control[2]**		**FMT[3]**		**S.E.M.**	**P-value**		
Offspring treatment	**Control[4]**	**Inulin[5]**	**Control**	**Inulin**		**Interaction**	**Sow**	**Offspring**
Weight (kg)								
Birth	1.52	1.56	1.30	1.31	0.639	0.99	0.85	0.99
Weaning	9.1	9.2	7.5	7.5	0.64	0.62	0.18	0.97
Day 100	59.0[a]	58.6[a]	51.2[b]	53.7[a,b]	1.31	<0.001	<0.001	0.41
Day 140	104.9[a]	103.9[a]	94.3[b]	96.8[b]	1.29	<0.001	<0.001	0.59
ADFI[6] (g/day)	2022	1975	1904	1955	41.1	0.25	0.13	0.96
ADG[7] (g/day)	831	806	800	828	15.4	0.56	0.63	0.93
FCE[8] (g/g)	2.39	2.37	2.31	2.37	0.075	0.56	0.63	0.77
RFI[9] (g/day)								
Weaning to day 140	13.7[a,b]	25.2[a]	-13.9[b]	-20.9[b]	15.96	0.05	0.02	0.88
Carcass traits								
Weight (kg)	81.0[a]	80.1[a]	72.1[b]	75.0[b]	1.62	0.01	<0.001	0.54
Kill out yield (%)	76.9	76.3	76.8	77.7	0.65	0.25	0.33	0.83
Fat depth (mm)	13.6	13.6	14.1	13.4	0.41	0.36	0.76	0.41
Muscle depth (mm)	53.5[a,b]	52.1[b]	52.5[a,b]	53.8[a]	0.71	0.05	0.61	0.92
Lean meat yield (%)	56.4	56.7	56.4	56.6	0.36	0.93	0.87	0.47

[1]Least square means and pooled standard error of the mean are presented.
Sows: [2]Control (n=11) and [3]FMT procedure (FMTP; n=11); Piglets: [4]Control (n=62), [5]Inulin (n=59) for the first 6 weeks post-weaning.
Days in the table correspond to days of age. [6]ADFI: average daily feed intake (between weaning and ~ day 140 of age); [7]ADG: average daily gain (between weaning and ~ day 140 of age); [8]FCE: feed conversion efficiency (between weaning and ~ day 140 of age); [9]RFI: residual feed intake.
[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P \leq 0.05$).

Table 5.2. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on haematological parameters in pigs[1]

Measure	Control[2]		FMT[3]		S.E.M.	P-value		
	Control[4]	Inulin[5]	Control	Inulin		Interaction	Sow	Offspring
White blood cells ($\times 10^3$ cells/µL)	28.4[a]	21.6[b]	24.7[a,b]	26.2[a,b]	1.54	0.01	0.78	0.09
Lymphocytes								
%	31.5	39.9	33.6	34.2	2.72	0.16	0.52	0.11
no. $\times 10^3$ cells/µL	8.9	8.0	8.3	8.6	0.59	0.32	0.95	0.64
Monocytes								
%	3.30	4.24	3.01	2.61	0.663	0.32	0.16	0.69
no. x 10^3 cells/µL	0.94	0.89	0.74	0.74	0.200	0.90	0.39	0.90
Granulocytes								
%	65.2	56.6	63.4	51.5	3.65	0.66	0.35	0.01
no. $\times 10^3$ cells/µL	18.6	12.1	15.7	11.7	1.48	0.41	0.26	0.001
Red blood cells ($\times 10^6$ cells/µL)	7.40	7.33	7.39	7.20	0.199	0.77	0.73	0.52
Red cell distribution width (fL)	19.0	19.2	20.5	20.1	0.80	0.69	0.14	0.93
Haemoglobin (g/dL)	13.9[a,b]	13.6[a,b]	12.7[b]	14.0[a]	0.39	0.05	0.31	0.16
Haematocrit (%)	0.42	0.42	0.40	0.39	0.015	0.96	0.13	0.87
Mean corpuscular volume (fL)	56.7	56.6	54.0	55.9	1.01	0.32	0.11	0.37
Mean corpuscular haemoglobin								
%	18.8	18.9	17.2	18.4	0.45	0.24	0.03	0.16
pg	33.1	32.8	31.5	32.7	0.49	0.15	0.09	0.34
Platelets ($\times 10^6$ cells /µL)	307	207	241	272	51.6	0.43	0.98	0.42
Mean platelet volume (fL)	9.3	10.3	9.7	10.2	0.28	0.44	0.63	0.01

[1]Least square means and pooled standard error of the mean are presented. Sows: [2]Control (n=11) and [3]FMT procedure (FMTP; n=11); Piglets: [4]Control (n=16), [5]Inulin (n=16) for the first 6 weeks post-weaning. [a,b,c] Within each row, values that do not share a common superscript are significantly different ($P \leq 0.05$).

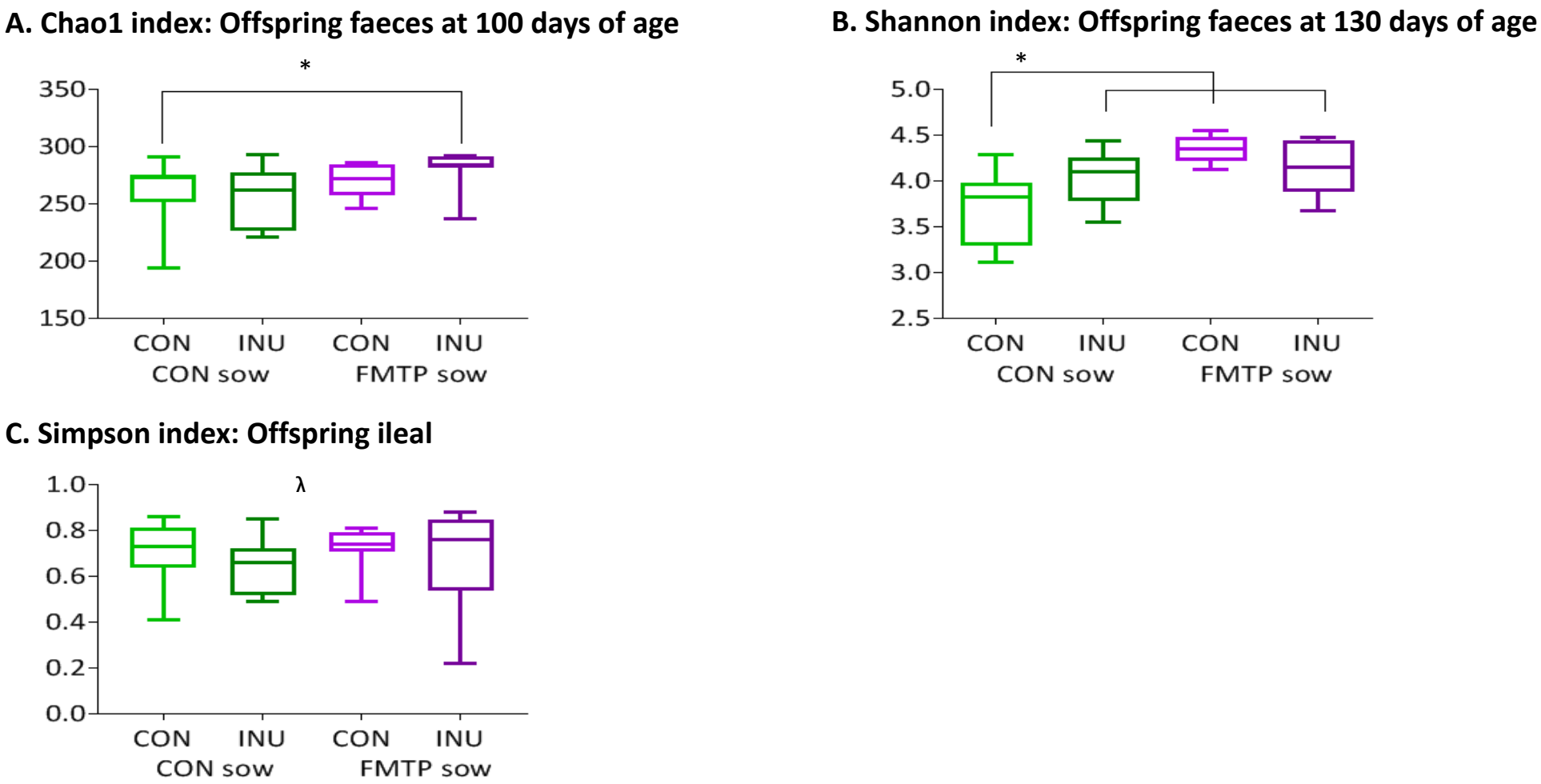
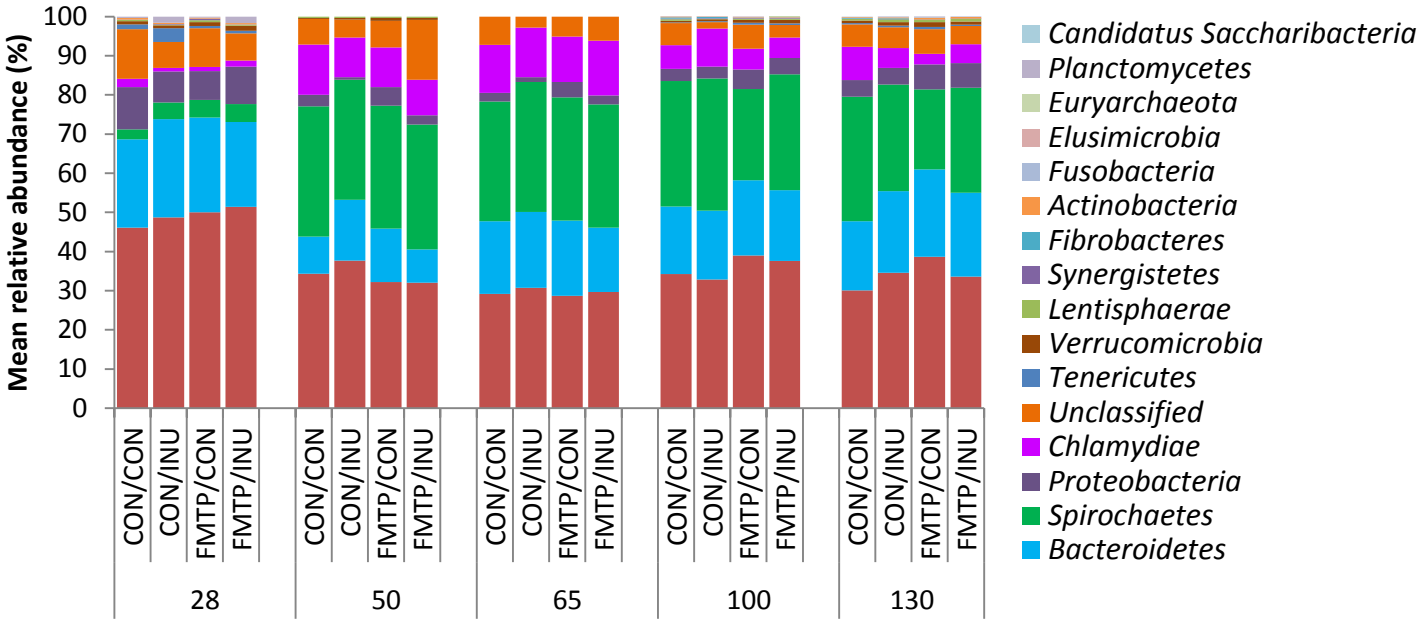


Figure 5.1. Variations in α-diversity (at the genus level) of the offspring microbiota in A. Faeces at 100 days of age, B. Faeces at 130 days of age and C. Ileal digesta as a result of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning
Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTP) n=16; Offspring treatment level: Control (CON) n=16; Inulin (INU) n=16.
*Indicates significant differences at sow × offspring treatment level ($P<0.05$); φ indicates sow treatment effect ($P\leq0.05$); λ indicates offspring treatment effect ($P\leq0.05$).

A. Faeces



B. Digesta

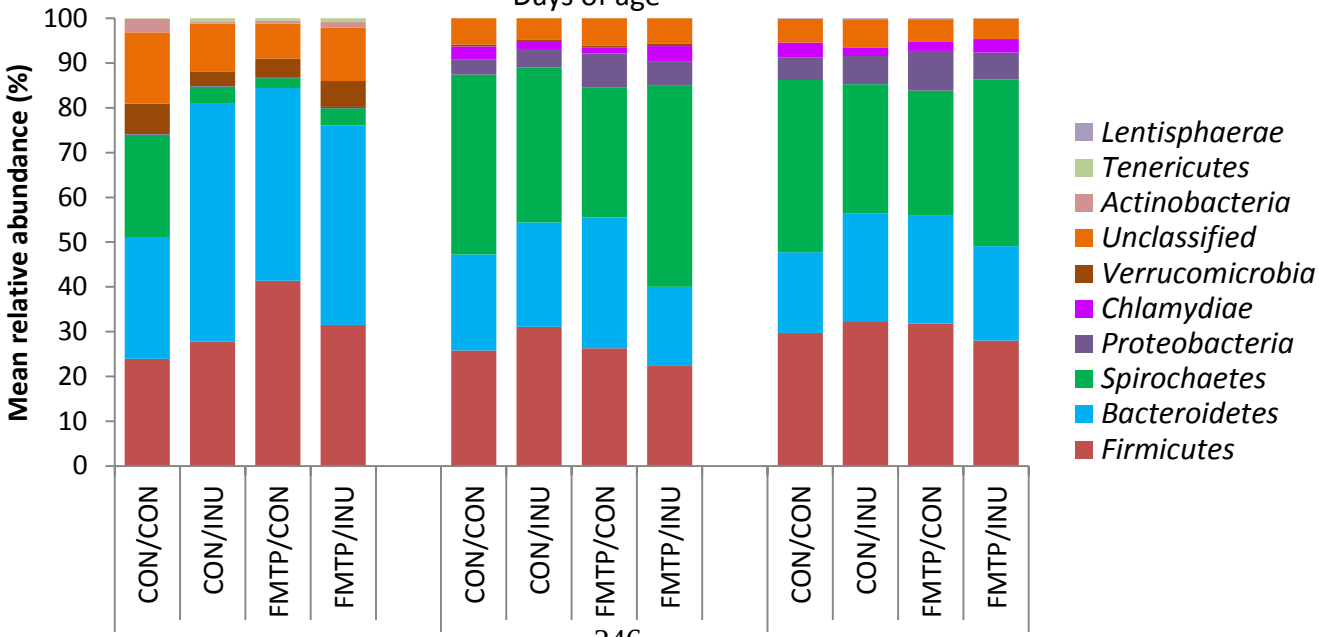


Figure 5.2. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on microbial composition at the phylum level in offspring from A. faeces at 28, 50, 65, 100 and 130 days old, and B. digesta from ileum, caecum and colon

Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTP) n=16; Offspring treatment level: Control (CON) n=16; Inulin (INU) n=16.

Bars represent mean relative abundance of phyla detected for each of the treatments.

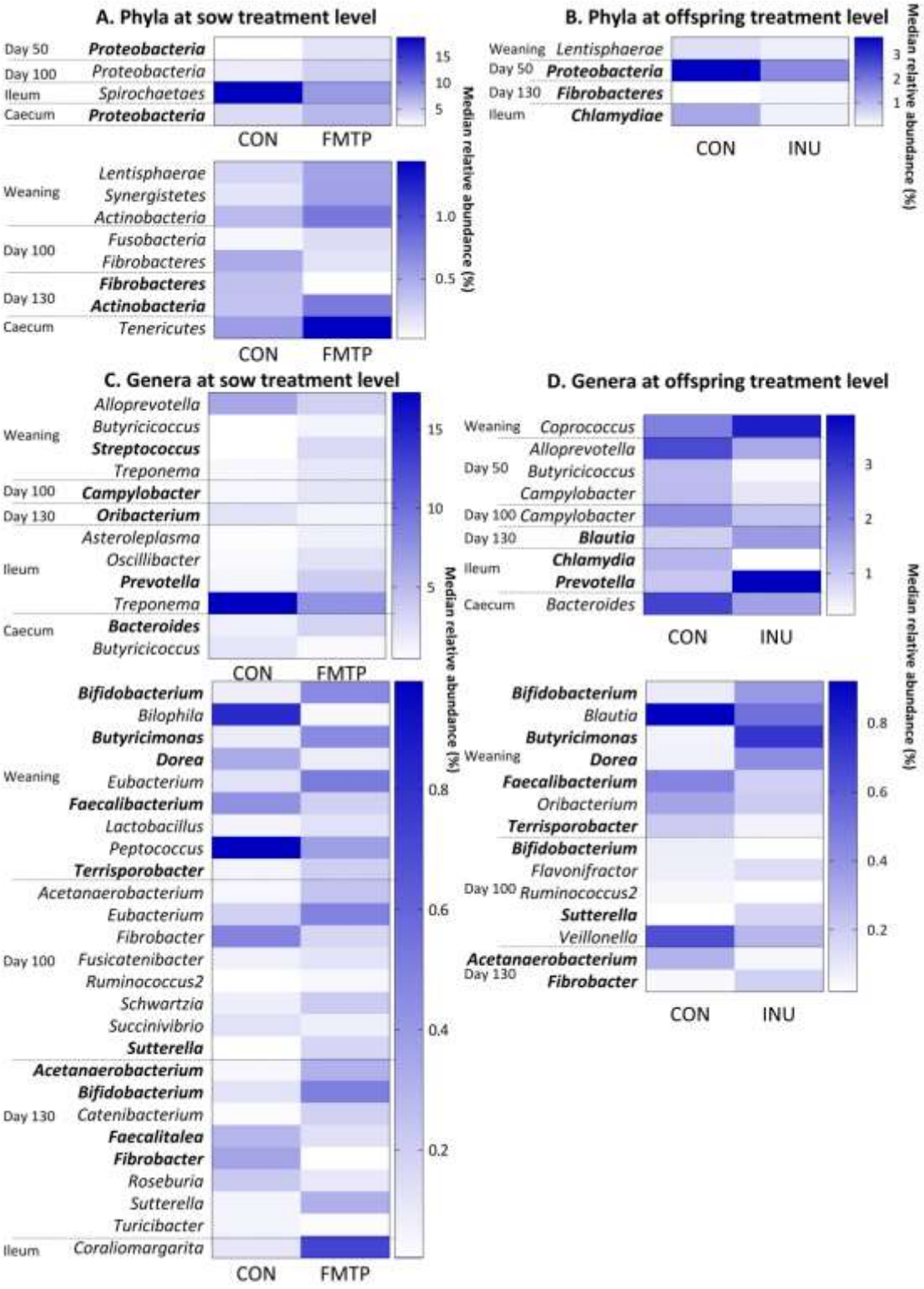

A. Phyla at sow treatment level
Day 50 Proteobacteria
Day 100 Proteobacteria
Ileum Spirochaetaes
Caecum Proteobacteria
15
10
5
CON
FMTP
Weaning Lentisphaerae
Synergistetes
Actinobacteria
Day 100 Fusobacteria
Fibrobacteres
Day 130 Fibrobacteres
Actinobacteria
Caecum Tenericutes
1.0
0.5
Median relative abundance (%)
B. Phyla at offspring treatment level
Weaning Lentisphaerae
Day 50 Proteobacteria
Day 130 Fibrobacteres
Ileum Chlamydiae
3
2
1
CON
INU
Median relative abundance (%)
C. Genera at sow treatment level
Weaning Alloprevotella
Butyricicoccus
Streptococcus
Treponema
Day 100 Campylobacter
Day 130 Oribacterium
Ileum Asteroleplasma
Oscillibacter
Prevotella
Treponema
Caecum Bacteroides
Butyricicoccus
15
10
5
CON
FMTP
Median relative abundance (%)
Weaning Bifidobacterium
Bilophila
Butyricimonas
Dorea
Eubacterium
Faecalibacterium
Lactobacillus
Peptococcus
Terrisporobacter
Day 100 Acetanaerobacterium
Eubacterium
Fibrobacter
Fusicatenibacter
Ruminococcus2
Schwartzia
Succinivibrio
Sutterella
Day 130 Acetanaerobacterium
Bifidobacterium
Catenibacterium
Faecalitalea
Fibrobacter
Roseburia
Sutterella
Turicibacter
Ileum Coraliomargarita
0.8
0.6
0.4
0.2
CON
FMTP
Median relative abundance (%)
D. Genera at offspring treatment level
Weaning Coprococcus
Alloprevotella
Day 50 Butyricicoccus
Campylobacter
Day 100 Campylobacter
Day 130 Blautia
Ileum Chlamydia
Prevotella
Caecum Bacteroides
3
2
1
CON
INU
Median relative abundance (%)
Bifidobacterium
Blautia
Butyricimonas
Weaning Dorea
Faecalibacterium
Oribacterium
Terrisporobacter
Bifidobacterium
Flavonifractor
Day 100 Ruminococcus2
Sutterella
Veillonella
Acetanaerobacterium
Day 130 Fibrobacter
0.8
0.6
0.4
0.2
CON
INU
Median relative abundance (%)

Figure 5.3. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on median relative abundance (%) of bacterial phyla in faeces and digesta at A. sow treatment level and B. offspring treatment level and of bacterial genera at C. sow treatment level and D. offspring treatment level

Data from 32 pigs: Sow treatment level: control n=16; FMT procedure (FMTP) n=16; Offspring treatment level: Control n=16; Inulin n=16.

Heat maps are split by relative abundance with higher abundance phyla/genera shown in the upper heat maps, and lower abundance phyla/genera shown in the lower heat maps.

Additional sow treatment × offspring treatment interactions not shown in either panel A, B, C or D given in Table S5.3.

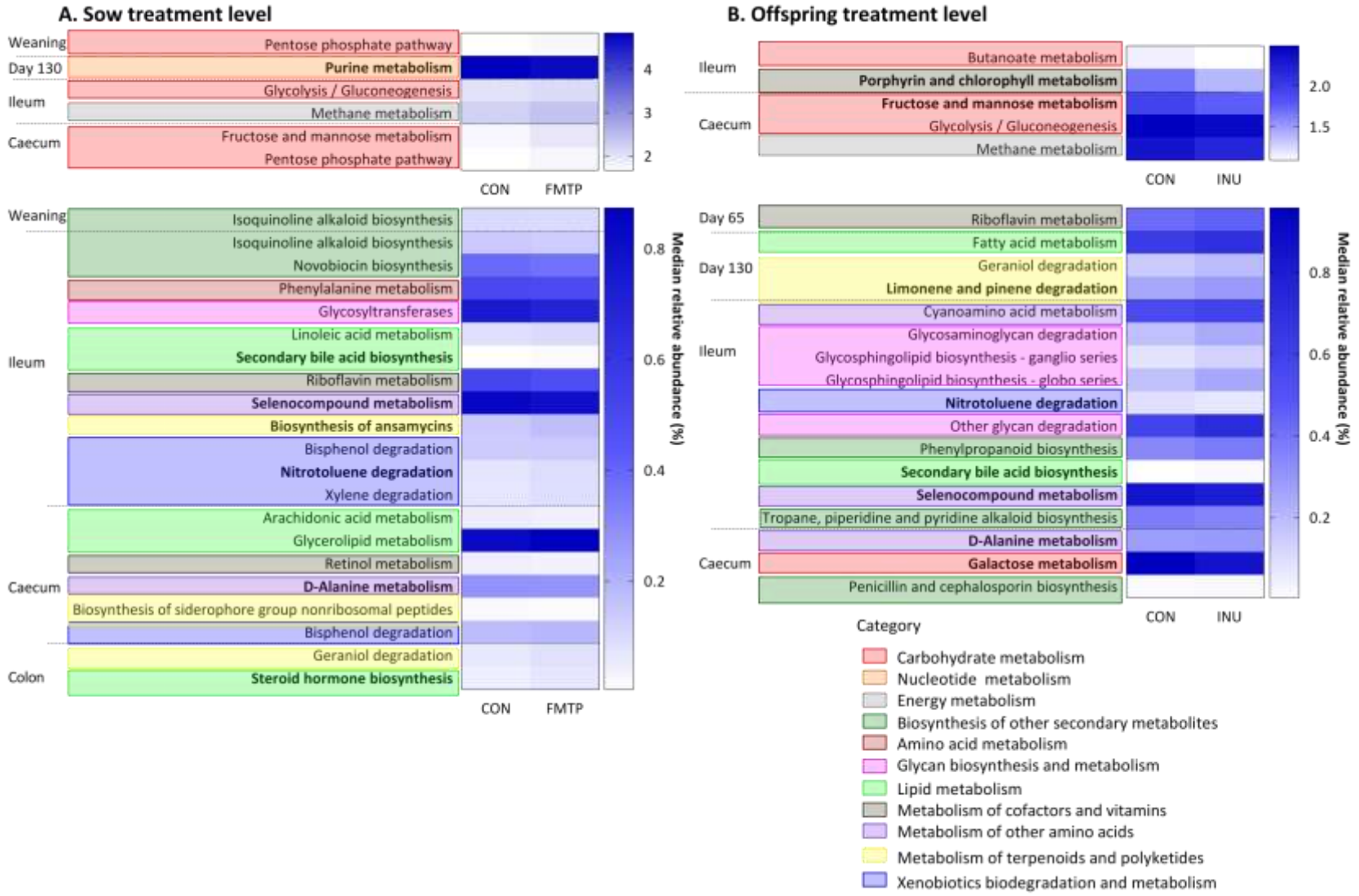

A. Sow treatment level
Weaning
Day 130
Ileum
Caecum
Pentose phosphate pathway
Purine metabolism
Glycolysis / Gluconeogenesis
Methane metabolism
Fructose and mannose metabolism
Pentose phosphate pathway
CON
FMTP
4
3
2
Weaning
Ileum
Caecum
Colon
Isoquinoline alkaloid biosynthesis
Isoquinoline alkaloid biosynthesis
Novobiocin biosynthesis
Phenylalanine metabolism
Glycosyltransferases
Linoleic acid metabolism
Secondary bile acid biosynthesis
Riboflavin metabolism
Selenocompound metabolism
Biosynthesis of ansamycins
Bisphenol degradation
Nitrotoluene degradation
Xylene degradation
Arachidonic acid metabolism
Glycerolipid metabolism
Retinol metabolism
D-Alanine metabolism
Biosynthesis of siderophore group nonribosomal peptides
Bisphenol degradation
Geraniol degradation
Steroid hormone biosynthesis
CON
FMTP
0.8
0.6
0.4
0.2
Median relative abundance (%)
B. Offspring treatment level
Ileum
Caecum
Butanoate metabolism
Porphyrin and chlorophyll metabolism
Fructose and mannose metabolism
Glycolysis / Gluconeogenesis
Methane metabolism
CON
INU
2.0
1.5
Day 65
Day 130
Ileum
Caecum
Riboflavin metabolism
Fatty acid metabolism
Geraniol degradation
Limonene and pinene degradation
Cyanoamino acid metabolism
Glycosaminoglycan degradation
Glycosphingolipid biosynthesis - ganglio series
Glycosphingolipid biosynthesis - globo series
Nitrotoluene degradation
Other glycan degradation
Phenylpropanoid biosynthesis
Secondary bile acid biosynthesis
Selenocompound metabolism
Tropane, piperidine and pyridine alkaloid biosynthesis
D-Alanine metabolism
Galactose metabolism
Penicillin and cephalosporin biosynthesis
CON
INU
0.8
0.6
0.4
0.2
Median relative abundance (%)
Category
Carbohydrate metabolism
Nucleotide metabolism
Energy metabolism
Biosynthesis of other secondary metabolites
Amino acid metabolism
Glycan biosynthesis and metabolism
Lipid metabolism
Metabolism of cofactors and vitamins
Metabolism of other amino acids
Metabolism of terpenoids and polyketides
Xenobiotics biodegradation and metabolism

Figure 5.4. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on predicted functional pathways for offspring faecal and intestinal microbiota at A. sow and B. offspring treatment level

Data from 32 pigs: Sow treatment level: control n=16; FMT procedure (FMTP) n=16; Offspring treatment level: Control n=16; Inulin n=16.

Pathways are from the KEGG database and level 3 pathways are presented. Median relative abundance for pathways differing between offspring, due to a sow or offspring treatment effect are represented in the heat maps.

Additional sow treatment × offspring treatment interactions not shown in either panel A, B, C or D given in Table S5.3.

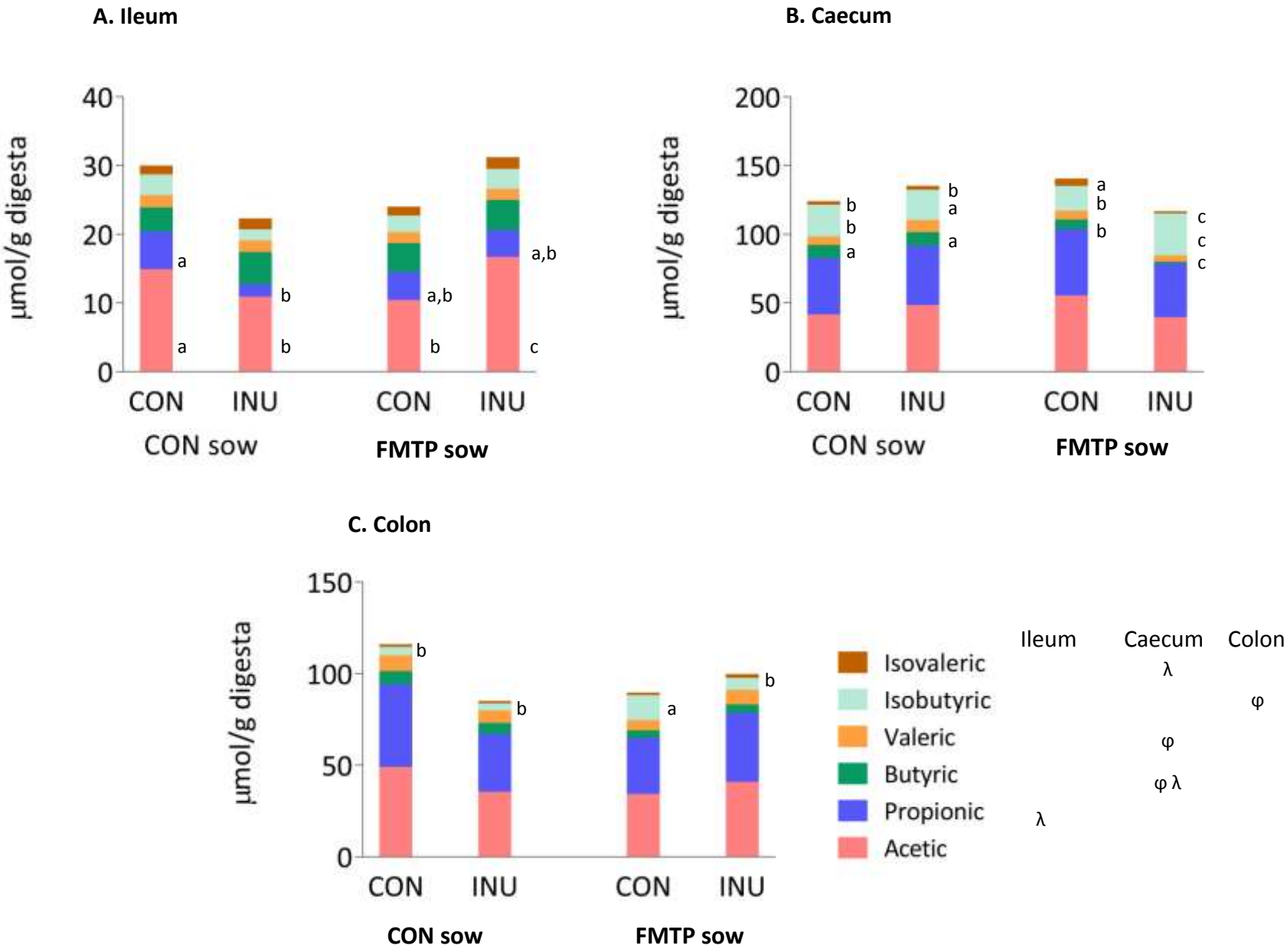

A. Ileum
B. Caecum
C. Colon
µmol/g digesta
CON
INU
CON sow
FMTP sow
Isovaleric
Isobutyric
Valeric
Butyric
Propionic
Acetic
Ileum
Caecum
Colon
λ
φ
φ
φ λ
λ

Figure 5.5. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on volatile fatty acid concentrations in A. Ileum; B. Caecum; C. Colon at 140 days old pigs

Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTP) n=16; Offspring treatment level: Control (CON) n=16; Inulin (INU) n=16.

[a,b,c] Within each volatile fatty acid, bars that do not share a common superscript are significantly different due to a sow x offspring treatment interaction ($P \leq 0.05$); φ indicates sow treatment effect ($P \leq 0.05$); λ indicates offspring treatment effect ($P \leq 0.05$).

Ileum: propionic acid was affected by offspring treatment (CON 4.7, INU 2.7 µmol/g)

Caecum: butyric acid was affected by sow treatment (CON 9.6, FMT 3.2 µmol/g), and offspring treatment (CON 8.1, INU 3.9 µmol/g), valeric acid was affected by sow treatment (CON 7.4, FMT 5.6 µmol/g), and isovaleric acid was affected by offspring treatment (CON 3.6, INU 2.1 µmol/g).

Colon: isobutyric acid was affected by sow treatment (CON 4.2, FMT 9.4 µmol/g).

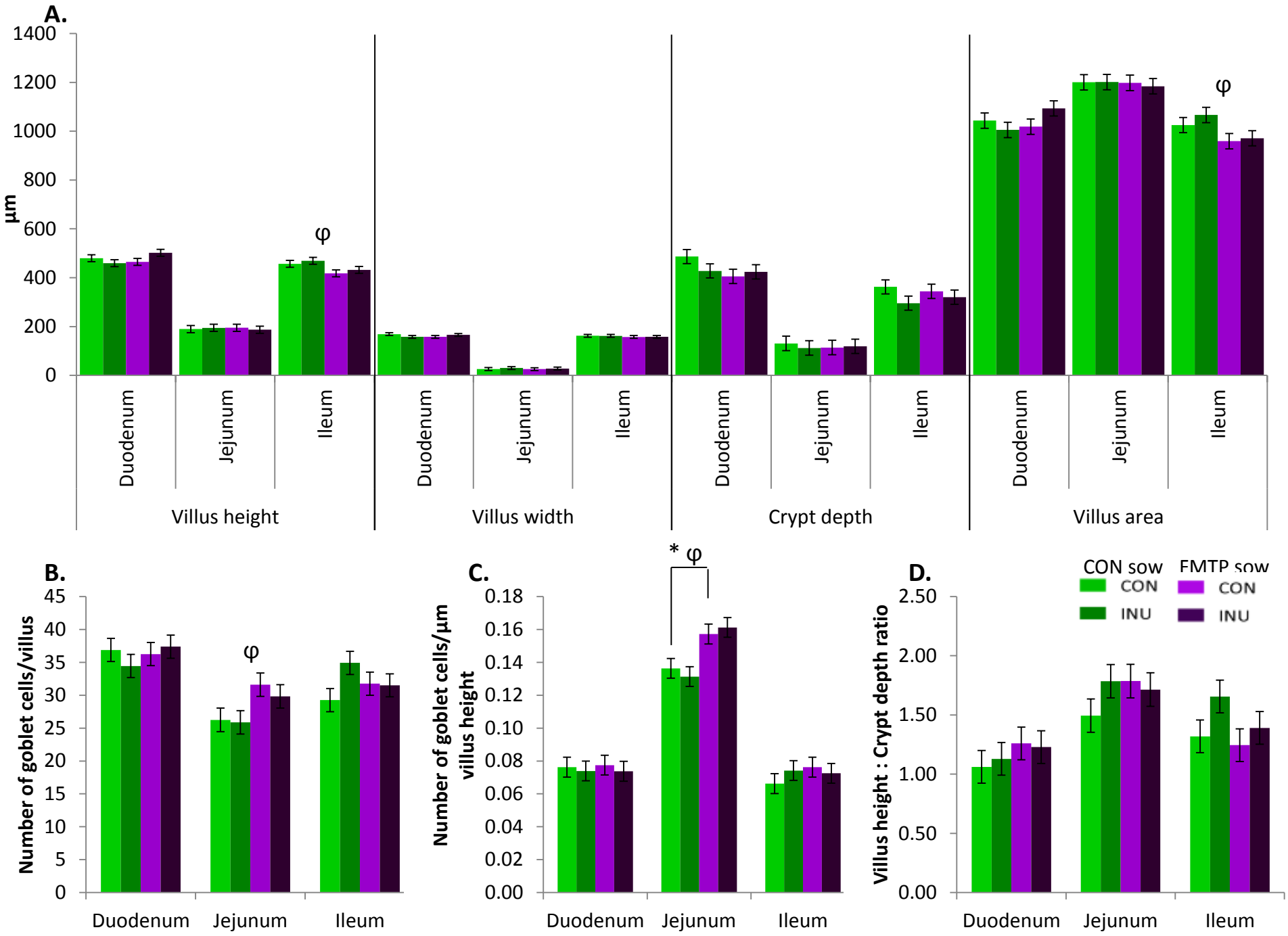

A.
µm
1400
1200
1000
800
600
400
200
0
φ
φ
Duodenum
Jejunum
Ileum
Villus height
Villus width
Crypt depth
Villus area
B.
Number of goblet cells/villus
45
40
35
30
25
20
15
10
5
0
φ
Duodenum
Jejunum
Ileum
C.
Number of goblet cells/µm villus height
* φ
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Duodenum
Jejunum
Ileum
D.
CON sow
FMTP sow
CON
INU
CON
INU
Villus height : Crypt depth ratio
2.50
2.00
1.50
1.00
0.50
0.00
Duodenum
Jejunum
Ileum

Figure 5.6. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on intestinal histology: A. Villus height, width, crypt depth and villus area, B. Number goblet cells per villus, C. Number of goblet cells per µm villus height, and D. Villus height to crypt depth ratio

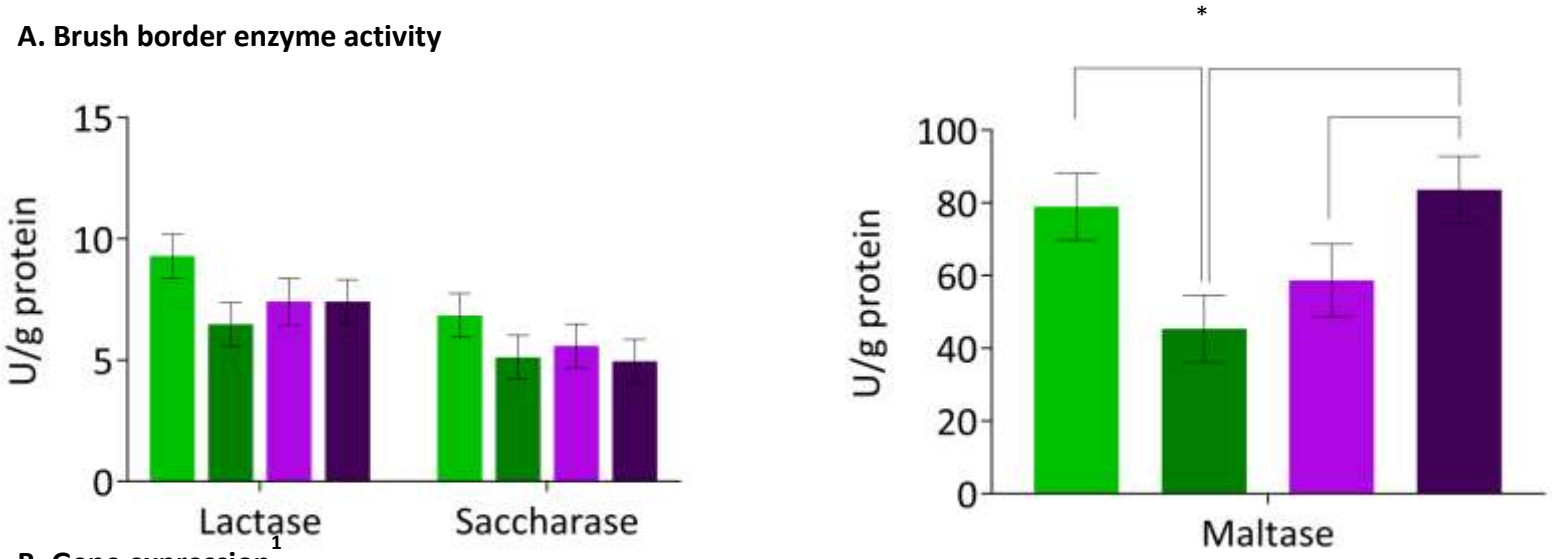
Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTP) n=16; Offspring treatment level: Control (CON) n=16; Inulin (INU) n=16.

*Indicates significant differences at sow × offspring treatment level ($P \leq 0.05$); φ indicates sow treatment effect ($P \leq 0.05$); λ indicates offspring treatment effect ($P \leq 0.05$).

Jejunum: number of goblet cells (CON: 26 vs. FMT: 31) and number of goblet cells per µm villus height (CON: 0.13 vs. FMT: 0.16) were affected by sow treatment ($P \leq 0.05$).

Ileum: villus area (CON: 1046 vs. FMT: 965 µm) was affected by sow treatment ($P < 0.05$), and villus height to crypt depth ratio (CON: 1.28 vs. INU: 1.52) tended to be affected by offspring treatment ($P = 0.09$).

A. Brush border enzyme activity



B. Gene expression

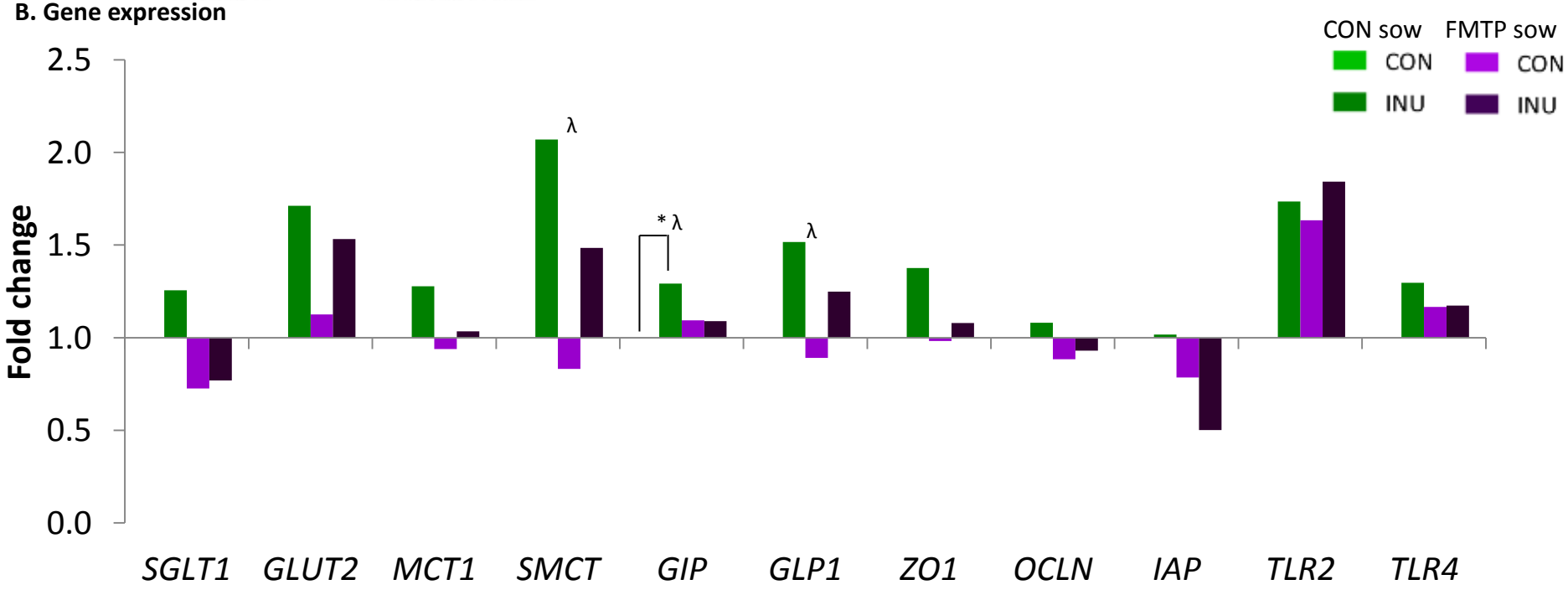


Figure 5.7. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on A. brush border enzyme activity and on B. expression of 11 selected genes in the duodenal mucosa of 140 day-old offspring

Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT n=16; Offspring treatment level: Control (CON) n=16; Inulin (INU) n=16.

*Indicates significant differences at sow × offspring treatment level ($P<0.05$); φ indicates sow treatment effect ($P<0.05$); λ indicates offspring treatment effect ($P<0.05$).

[1]Bars represent log_{10}-fold changes relative to Control sow × Control offspring treatment after normalization to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Beta-actin (ACTB) and Beta-2 microglobulin (B2M) gene expression.

Candidate genes measured: sodium-dependent glucose transporter 1 (SGLT1), monocarboxylate transporter 1 (MCT1), sodium-coupled monocarboxylate transporter (SMCT), intestinal alkaline phosphatase (ALPi), tight-junction proteins [zona occludens 1 (ZO1) and occludin (OCLN)], toll-like receptor 2 (TLR2) and 4 (TLR4), facilitated glucose transporter member 2 (GLUT2), glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP1).

Gene expression affected by offspring treatment: *GLP1* (CON: 0.94, INU:1.38 fold-change); *GIP* (CON: 1.05, INU:1.19 fold-change); *SMCT* (CON: 0.91, INU:1.77 fold-change); and *ZO1* (CON: 0.99, INU:1.23 fold-change; P=0.06).

5.9. Supplementary Information

Table S5.1. Composition of all diets used in the study (g/Kg)

Diet Type	Starter		Link		Weaner		Finisher	Sow	
	Control	Inulin	Control	Inulin	Control	Inulin		Pregnant	Lactation
Barley	0.0	0.0	0.0	0.0	24.8	25.3	38.5	89.7	34.9
Wheat	22.2	19.1	39.9	36.9	43.1	36.9	40.4	0.0	43.2
Maize	8.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Soya	16.9	19.7	22.9	24.7	20.0	21.4	17.5	7.0	15.0
Full fat Soya	10.0	7.0	7.0	5.0	5.0	5.0	0.0	0.0	0.0
Inulin	0.0	2.0	0.0	2.0	0.0	3.0	0.0	0.0	0.0
Lactofeed 70[1]	20.0	20.0	20.0	20.0	0.0	0.0	0.0	0.0	0.0
Skim milk powder	12.5	12.5	5.0	5.0	0.0	0.0	0.0	0.0	0.0
Soya oil	7.5	8.8	2.5	3.6	4.0	5.3	10.0	10.0	40.0
Lysine HCl (78.8)	0.5	0.4	0.4	0.4	0.5	0.43	4.0	1.0	3.5
DL-Methionine	0.3	0.4	0.2	0.3	0.2	0.2	1.0	1.0	1.0
L-Threonine (98)	0.2	0.2	0.2	0.02	0.2	0.2	1.5	0.0	1.0
L-Tryptophan	0.09	0.09	0.05	0.06	0.02	0.02	0.0	0.0	0.0
Vitamin and mineral mix	0.3	0.3	0.3	0.3	0.3	0.3	1.0	1.5	1.5
Natuphos 5000 FTU/g[5]	0.01	0.01	0.01	0.01	0.01	0.01	0.1	0.1	0.1
Salt feed grade	0.3	0.3	0.3	0.3	0.3	0.3	3.0	4.0	4.0
Dicalcium phosphate	0.1	0.1	0.1	0.1	0.6	0.6	2.0	5.0	5.0
Limestone flour	1.1	1.1	1.1	1.1	0.9	0.9	13.0	11.0	12.0
Chemical analysis (g/Kg DM)									
Crude protein	235.9	252.8	211.0	205.4	195.7	172.1	161	119	148
Crude fibre	19.8	23.4	34.8	37.5	38.9	31.7	24	32	25
Crude ash	66.2	63.5	48.3	45.4	44.6	49.8	41	37	41
Ether extract	114.7	57.9	70.7	27.2	33.2	63.4	27	29	63
DE MJ/kg[6]	17.9	17.0	16.9	16.0	15.9	16.3	16.3	15.6	16.8
NE (IFIP)[7]	11.43	10.25	10.55	9.78	9.50	10.45	9.8	9.5	10.5
Amino acids (g/Kg)									
Lysine	16.2	15.0	13.0	11.1	6.4	9.9	11.50	6.50	9.90
Methionine	6.8	5.7	4.5	3.6	2.1	3.4	3.70	2.00	3.40
Meth + Cyst	9.7	9.0	7.9	6.8	4.7	6.4	7.40	5.00	6.80
Threonine	10.5	9.8	8.7	7.5	4.5	6.5	7.90	4.60	6.70
Tryptophan	3.6	3.3	2.6	2.2	1.6	2.0	1.50	1.20	1.40

[1]Lactofeed 70 contains 70% lactose, 11.5% protein, 0.5% oil, 7.5% ash and 0.5% fibre (Volac, Cambridge, UK).
[2]Premix provided per Kg of complete diet: Copper sulphate $7H_2O$, 62 g; Ferrous sulphate monohydrate, 450 g; Manganese oxide, 60 g; Zinc oxide, 150 g; Potassium iodate, 1 g; Sodium selenite, 0.6 g; Cu, 155 mg; Fe, 90 mg; Mn, 47 mg; Zn, 120 mg, I, 0.6 mg; Se, 0.3 mg; vitamin A, 6000 IU; vitamin $D_{3,}$ 1000 IU; vitamin E, 100 IU; vitamin K, 4 mg; vitamin $B_{12,}$ 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin $B_{1,}$ 2 mg; vitamin $B_{6,}$ 3 mg; Endox, 60 g.
[3] Premix provided per Kg of complete diet: Copper sulphate $7H_2O$, 60 g; Ferrous sulphate monohydrate, 120 g; Manganese oxide, 40 g; Zinc oxide, 100 g; Potassium iodate, 0.5 g; Sodium selenite, 0.4 g; Cu, 15 mg; Fe, 24 mg; Mn, 31 mg; Zn, 80 mg, I, 0.3 mg; Se, 0.2 mg; vitamin A, 2000 IU; vitamin $D_{3,}$ 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin $B_{12,}$ 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin $B_{1,}$ 2 mg; vitamin $B_{6,}$ 3 mg.
[4] Premix provided per Kg of complete diet: Copper sulphate $7H_2O$, 60 g; Ferrous sulphate monohydrate, 200 g; Manganese oxide, 80 g; Zinc oxide, 100 g; Potassium iodate, 1 g; Sodium selenite, 0.4 g; Cu, 15 mg; Fe, 70 mg; Mn, 62 mg; Zn, 80 mg, I, 0.6 mg; Se, 0.2 mg; vitamin A, 1000 IU; vitamin $D_{3,}$ 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; vitamin $B_{12,}$ 15 µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; Biotin, 200 mg; Folic acid, 5 g; vitamin $B_{1,}$ 2 mg; vitamin $B_{6,}$ 3 mg.
[5] Phytase: Natuphos – BASF; 5000 FTU/gm equal to 500 FTU per Kg finished feed.
[6]Digestible energy is megajoules per kilogram of dry matter (DE= 4168-9.1*ASH +1.9*CP+3.9*EE-3.6*NDF)
[7]Calculated values.

Table S5.2. Forward and reverse primers used for quantitative PCR, PCR efficiency, and coefficient correlation of standard curves

Gene symbol[1]	Accession number[2]	Gene name	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)	Ref[3]	Eff. (%)[4]	Corr.[5]
ACTB	XM_003357928.2	Beta-actin	GGGCATCCTGACCCTCAAG	TGTAGAAGGTGTGATGCCAGATCT	89	1	97.3	0.99
B2M	NM_213978.1	Beta-2-microglobulin	CCCCCGAAGGTTCAGGTT	GCAGTTCAGGTAATTTGGCTTTC	66	1	102.2	0.99
GAPDH	NM_001206359.1	Glyceraldehyde-3-phosphate dehydrogenase	GGCGTGAACCATGAGAAGTATG	GGTGCAGGAGGCATTGCT	60	1	96.5	0.99
HPRT1	NM_001032376.2	Hypoxanthine guanine phosphoribosyl transferase	AGAAAAGTAAGCAGTCAGTTTCATATCAGT	ATCTGAACAAGAGAGAAAATACAGTCAATAG	131	1	92.1	0.99
OAZ1	NM_001122994.2	Ornithine decarboxylase antizyme 1	TCGGCTGAATGTAACAGAGGAA	GAGCCTGGATTGGACGTTTAAA	70	1	99.2	0.99
OCLN	NM_001163647.2	Occludin	TTGTGGGACAAGGAACGTATTTA	TGCCTGCCGACACGTTT	76	1	95.4	0.98
ZO1	XM_013993251.1	Zona occludin 1	AAGCCCTAAGTTCAATCACAATCT	ATCAAACTCAGGAGGCGGC	131	1	109.2	0.98
SGLT1 (SLC5A1)	NM_001164021.1	Sodium-dependent glucose transporter 1	TGTCTTCCTCATGGTGCCAA	AGGAGGGTCTCAGGCCAAA	149	1	108.0	0.99
GLUT2 (SLC2A2)	NM_001097417.1	Facilitated glucose transporter member 2	TACGGCATCTGCTAGCCTCAT	CCACCAATTGCAAAGATGGAC	66	2	89.3	1.00
MCT1 (SLC16A1)	AM286425.1	Monocarboxylate transporter 1	GGTGGAGGTCCTATCAGCAG	AAGCAGCCGCCAATAATCAT	74	1	96.4	1.00
SMCT (SLC5A12)	XM_003122908.1	Sodium-coupled monocarboxylate cotransporter	AGGTCTACCGCTTTGGAGCAT	GAGCTCTGATGTGAAGATGATGACA	77	2	82.3	0.99

Table S5.2. Forward and reverse primers used for quantitative PCR, PCR efficiency, and coefficient correlation of standard curves (continued)

Gene symbol[1]	Accession number[2]	Gene name	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)	Ref[3]	Eff. (%)[4]	Corr.[5]
GIP	NM_001287408.1	Glucose-dependent insulinotropic peptide	GGATGGTGGAGCAGTTGGA	CCAATCCTGAGCTGGGTTTG	71	2	98.1	0.99
GLP1	NM_001256594.1	Glucagon-like peptide-1	GCTGATGGTGGCGATCTTGT	TCCCAGCTCTTCCGAAACTC	69	2	98.1	0.99
TRL2	NM_213761.1	Toll-like receptor 2	AATAAGTTGAAGACGCTCCCAGAT	GTTGCTCCTTAGAGAAAGTATTGATCGT	97	1	92.7	0.99
TRL4	AB188301.2	Toll-like receptor 4	TGTGGCCATCGCTGCTAAC	GGTCTGGGCAATCTCATACTCA	124	1	105.8	0.98
ALPI	XM_003133729.3	Intestinal alkaline phosphatase	AGGAACCCAGAGGGACCATTC	CACAGTGGCTGAGGGACTTAGG	83	2	97.1	0.99

[1]Gene symbol: Alternate gene names are shown in brackets.
[2]Accession number: National Center for Biotechnology Information (NCBI) Entrez Gene (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene).
[3]Ref: references for oligonucleotide primer sequences. 1) Metzler-Zebeli BU, Mann E, Ertl R, Schmitz-Esser S, Wagner M, Klein D, Ritzmann M, Zebeli Q. Dietary calcium concentration and cereals differentially affect mineral balance and tight junction proteins expression in jejunum of weaned pigs. Br J Nutr. 2015; 113(7):1019-31. doi: 10.1017/S0007114515000380.; 2) Metzler-Zebeli BU, Ertl R, Grüll D, Molnar T, Zebeli Q. Enzymatically modified starch up-regulates expression of incretins and sodium-coupled monocarboxylate transporter in jejunum of growing pigs. Animal 2016; 11(7):1180-1188. Doi: 10.1017/S175131116002615.
[4]Eff: PCR efficiency: E = 10(-1/slope)-1.
[5]Corr: Correlation coefficient of standard curve.

Table S5.3. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on the relative abundance of bacterial composition and potential functionality[1]

Sow treatment	Control[2]		FMTP[3]		S.E.M.	P-value		
Offspring treatment	Control[4]	Inulin[5]	Control	Inulin		Interaction	Sow	Offspring
Weaning								
G_*Streptococcus*	0.73[b]	0.31[b]	2.24[a]	3.71[a]	0.134	<0.001	<0.001	0.68
G_*Butyricimonas*	0.04[b]	0.21[b]	0.08[b]	2.56[a]	0.018	<0.001	<0.001	<0.001
G_*Bifidobacterium*	0.09[b]	0.07[b]	0.10[b]	2.07[a]	0.027	0.001	0.04	0.03
G_*Faecalibacterium*	0.89[a]	0.20[b]	0.23[b]	0.16[b]	0.099	<0.001	0.003	0.001
G_*Dorea*	0.20[a,b]	0.57[a]	0.03[b]	0.31[a]	0.009	<0.001	0.01	<0.001
G_*Terrisporobacter*	0.13[a,b]	0.03[b]	0.31[a]	0.12[a,b]	0.012	0.003	0.007	0.005
Caffeine metabolism	0.0011[a]	0.0003[b]	0.0014[a]	0.0007[a,b]	0.00014	0.001	0.001	0.005
Day 50								
P_*Proteobacteria*	2.95[a]	1.10[b]	4.68[a]	2.65[a,b]	0.789	0.004	<0.001	<0.001
1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	0.0013[b]	0.0005[b]	0.0017[a,b]	0.0034[a]	0.00026	0.001	<0.001	0.61
Day 65								
Alpha-linolenic acid metabolism	0.0012[a,b]	0.0008[b]	0.0028[a]	0.0007[b]	0.00041	<0.001	0.15	<0.001
Day 100								
G_*Campylobacter*	1.07[b]	0.85[b]	3.22[a]	1.38[a,b]	0.567	<0.001	0.002	0.01
G_*Peptococcus*	0.08[b]	1.18[a]	0.95[a]	0.19[b]	0.032	<0.001	0.49	0.24
P_*Actinobacteria*	0.19	0.64	1.15	0.50	0.170	0.004	0.03	0.76
G_*Bifidobacterium*	0.03[a,b]	0.04[a,b]	0.23[a]	0.01[b]	0.004	0.006	0.39	0.05
G_*Sutterella*	0.002[b]	0.127[a]	0.158[a]	0.189[a]	0.0003	0.004	0.005	0.01

Table S5.3. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on the relative abundance of bacterial composition and potential functionality[1] (Continued)

Sow treatment	Control		FMTP		S.E.M.	P-value		
Offspring treatment	Control	Inulin	Control	Inulin		Interaction	Sow	offspring
Day 130								
G_*Sphaerochaeta*	18.00[a]	9.96[a,b]	4.51[b]	13.38[a,b]	2.442	0.008	0.07	0.41
G_*Oribacterium*	2.76[a]	1.80[a,b]	0.73[b]	1.80[a,b]	0.434	0.004	0.01	0.37
G_*Blautia*	1.39[a,b]	1.36[a,b]	0.61[b]	2.06[a]	0.374	0.006	0.13	0.02
G_*Faecalitalea*	0.42[a]	0.20[a,b]	0.06[b]	0.27[a,b]	0.031	0.001	0.02	0.29
P_*Fibrobacteres*	0.37[a]	0.35[a]	0.01[b]	0.39[a]	0.001	<0.001	<0.001	0.008
G_*Fibrobacter*	0.367[a]	0.351[a]	0.004[b]	0.092[a]	0.001	<0.001	<0.001	0.008
G_*Bifidobacterium*	0.03[b]	0.46[a,b]	0.82[a]	0.30[a,b]	0.007	0.004	0.02	0.15
G_*Acetanaerobacterium*	0.10[a,b]	0.02[b]	0.90[a]	0.11[a,b]	0.005	0.007	0.004	0.007
Limonene and pinene degradation	0.22[b]	0.31[a]	0.29[a]	0.27[a,b]	0.198	<0.001	0.14	0.01
Fatty acid metabolism	0.57[b]	0.72[a]	0.69[a,b]	0.66[a,b]	0.527	0.001	0.32	0.03
Purine metabolism	5.0[a]	4.7[b]	4.6[b]	4.8[b]	4.511	0.002	0.05	0.65
Ileum								
G_*Prevotella*	0.36[b]	3.35[a]	2.99[a]	4.54[a]	0.164	<0.001	0.004	0.002
G_*Sphaerochaeta*	2.78[a]	0.32[b]	0.30[b]	1.04[b]	0.143	<0.001	0.16	0.19
G_*Chlamydia*[6]	2.61[a]	0.20[b]	0.63[b]	0.30[b]	0.086	<0.001	0.22	<0.001
Glycosphingolipid biosynthesis - ganglio series	0.08	0.13	0.08	0.14	0.063	<0.001	0.76	<0.001
Glycosphingolipid biosynthesis - globo series	0.18	0.24	0.19	0.26	0.159	<0.001	0.36	<0.001
Nitrotoluene degradation	0.08	0.06	0.10	0.08	0.047	0.008	0.02	0.01
Biosynthesis of ansamycins	0.15	0.14	0.17	0.18	0.129	<0.001	<0.001	0.64
Secondary bile acid biosynthesis	0.002	0.012	0.008	0.019	0.0009	<0.001	0.001	<0.001
Porphyrin and chlorophyll metabolism	1.73	1.43	1.60	1.34	1.206	0.007	0.22	0.001
Selenocompound metabolism	0.86	0.82	0.81	0.78	0.755	<0.001	<0.001	0.002
Ether lipid metabolism	0.002	0.001	0.006	0.009	0.0005	<0.001	<0.001	0.64
1,2,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	0.002	0.001	0.001	0.002	0.0002	0.002	0.04	0.69

Table S5.3. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on the relative abundance of bacterial composition and potential functionality[1] (Continued)

Sow treatment	Control		FMTP		S.E.M.	P-value		
Offspring treatment	Control	Inulin	Control	Inulin		Interaction	Sow	offspring
Caecum								
P_*Proteobacteria*	3.79[b]	4.44[a,b]	7.50[a]	5.60[a,b]	2.832	0.009	0.003	0.95
G_*Bacteroides*	1.27[b]	1.57[b]	6.77[a]	1.58[b]	0.715	<0.001	0.006	0.04
G_*Flavonifractor*	0.09[b]	0.23[a,b]	0.34[a]	0.10[b]	0.052	0.002	0.27	0.66
Fructose and mannose metabolism	1.72[b]	1.79[b]	2.20[a]	1.74[b]	1.607	<0.001	0.002	0.006
Folate biosynthesis	0.57[b]	0.61[b]	0.67[s]	0.56[b]	0.533	<0.001	0.17	0.11
D-alanine metabolism	0.29[s]	0.28[s]	0.27[b]	0.29[s]	0.251	<0.001	<0.001	0.009
Phenylalanine, tyrosine and tryptophan biosynthesis	1.29[s,b]	1.36[a,b]	1.41[s]	1.22[b]	1.150	0.005	0.83	0.14
Streptomycin biosynthesis	0.43[a,b]	0.45[a,b]	0.49[a]	0.40[b]	0.373	0.004	0.86	0.08
Galactose metabolism	0.83[b]	0.89[a,b]	1.10[a]	0.82[a,b]	0.732	<0.001	0.07	0.05
N-glycan biosynthesis	0.028[b]	0.034[a,b]	0.041[a]	0.028[b]	0.0241	0.001	0.25	0.20
Colon								
P_*Lentisphaerae*	0.18[a,b]	0.42[a]	0.25[a]	0.06[b]	0.028	0.005	0.15	0.51
Steroid hormone biosynthesis	0.04[b]	0.05[a,b]	0.09[a]	0.05[a,b]	0.029	0.007	0.04	0.09
Retinol metabolism	0.027[b]	0.037[a,b]	0.047[a]	0.029[a,b]	0.0213	0.003	0.47	0.15
Glycine, serine and threonine metabolism	1.91[a]	1.88[a,b]	1.83[b]	1.92[a]	1.794	0.003	0.17	0.09
Glycosphingolipid biosynthesis-lacto and neolacto series	0.0005[b]	0.0008[a,b]	0.0011[a]	0.0003[c]	0.00029	<0.001	0.49	0.08

[1]Least square means and pooled standard error of the mean are presented. Sows: [2]Control (n=11) and [3]FMT procedure (FMTP; n=11); Piglets: [4]Control (n=16), [5]Inulin (n=16) for the first 6 weeks post-weaning.

[a,b,c] Within each row, values that do not share a common superscript are significantly different (P≤0.05).

Table S5.4. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on serum biochemical parameters

Sow treatment	Control[1]		FMT[2]		S.E.M[5]	P-value		
Offspring treatment	Control[3]	Inulin[4]	Control	Inulin		Interaction	Sow	Offspring
Blood urea nitrogen (mg/dL)	20.3	10.8	12.4	11.5	2.71	0.12	0.20	0.06
Total protein (g/L)	65.4	67.1	50.8	58.9	9.29	0.73	0.23	0.60
Triglycerides (mmol/L)	0.43	0.50	0.45	0.49	0.055	0.74	0.91	0.32
Glucose (mmol/L)	4.96	4.91	5.18	4.85	0.729	0.85	0.91	0.79
Cholesterol (mmol/L)	3.30^{A}	2.19^{B}	2.20^{B}	2.48^{B}	0.376	0.07	0.29	0.28
Creatine (µmol/L)	145.9	149.1	137.9	119.8	16.78	0.53	0.28	0.66
Creatinine kinase (µmol/L)	98.43	60.94	31.08	44.57	18.185	0.63	0.26	0.39

Sows were assigned to one of two treatment groups: [1]Control (n=11) and [2]FMT (n=11). FMT sows received FMT via gastric intubation on days 70 and 100 of gestation; Piglets were assigned to one of two treatment groups at weaning: [3]control; [4]Inulin for the first 6 weeks post-weaning (2% for 3 weeks and 3% for 3 weeks). [5]Least square means and pooled standard error of the mean are presented.

Data from 32 pigs: Sow treatment level control n=16; FMT n=16; Offspring treatment level control n=16; inulin n=16.

[A,B,C] Within each row, values that do not share a common superscript tend to be different (P≤010).

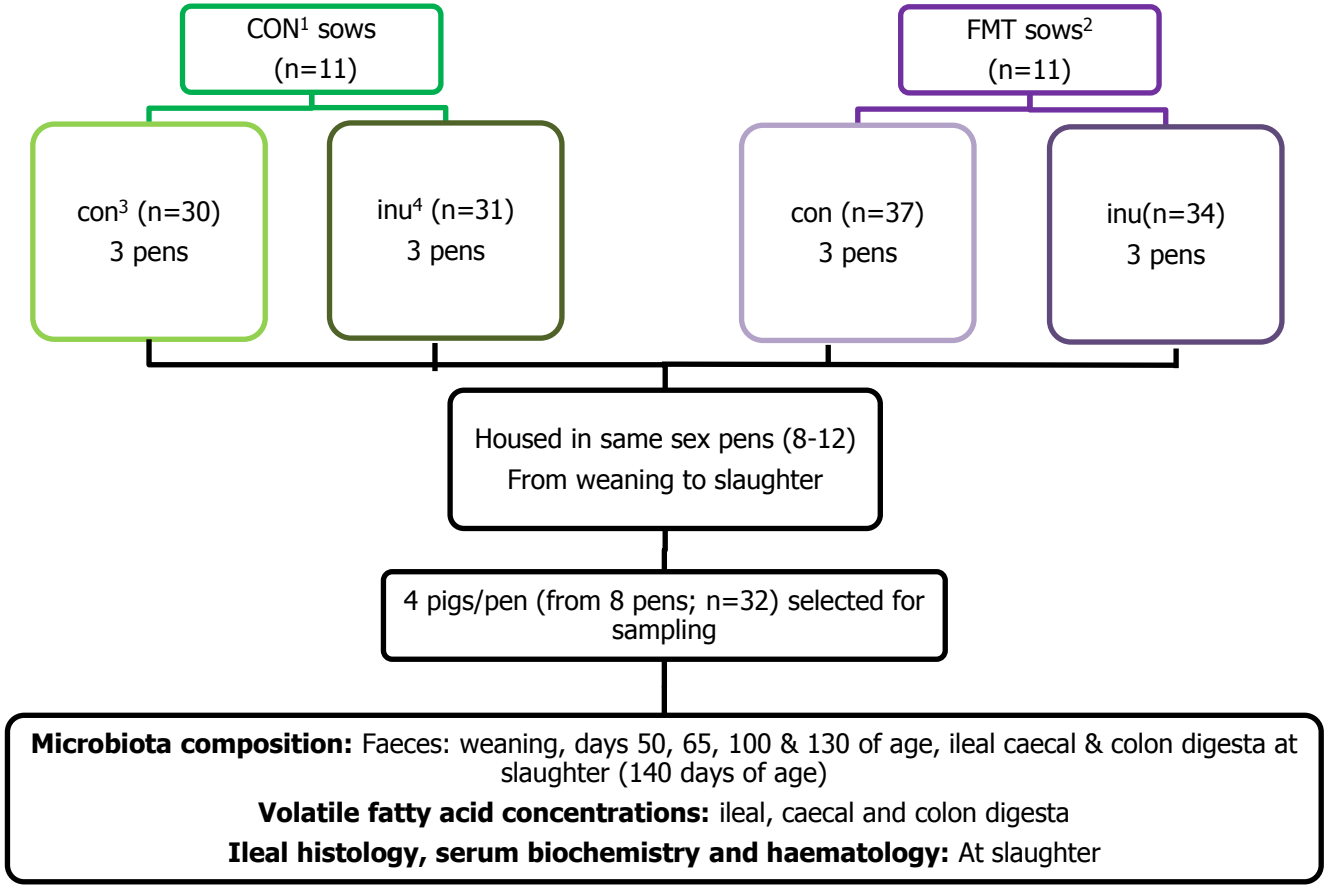

CON[1] sows
(n=11)
FMT sows[2]
(n=11)
con[3] (n=30)
3 pens
inu[4] (n=31)
3 pens
con (n=37)
3 pens
inu(n=34)
3 pens
Housed in same sex pens (8-12)
From weaning to slaughter
4 pigs/pen (from 8 pens; n=32) selected for sampling
Microbiota composition: Faeces: weaning, days 50, 65, 100 & 130 of age, ileal caecal & colon digesta at slaughter (140 days of age)
Volatile fatty acid concentrations: ileal, caecal and colon digesta
Ileal histology, serum biochemistry and haematology: At slaughter

Figure S5.1. Schematic depicting faecal inoculum preparation, sow treatment, offspring treatment and sample collection

Sows were assigned to one of two groups: [1]CON: control sows; [2]FMT, and were given a 1-week course of antibiotics from day 60 of gestation to kill off as much of the resident microbiota as possible, followed by a purgative and fasting for 36 h to empty the gastrointestinal tract. On days 70 and 100 of gestation, each sow was given 200 mL of thawed inoculum via gastric intubation as well as an anti-acid to prevent inactivation of the inoculum by gastric acid.

Piglets were weaned and assigned to [3]con: control or [4]inu: inulin for the first 6 weeks post-weaning (2 % for the first 3 weeks, and 3 % for the next 3 weeks); from 70 days of age all pigs were fed the same finisher diet until slaughter at 140 days of age.

32 pigs selected; Sow treatment level: Control, n=16; FMT, n=16; Pig treatment level: Control, n=16; Inulin, n=16

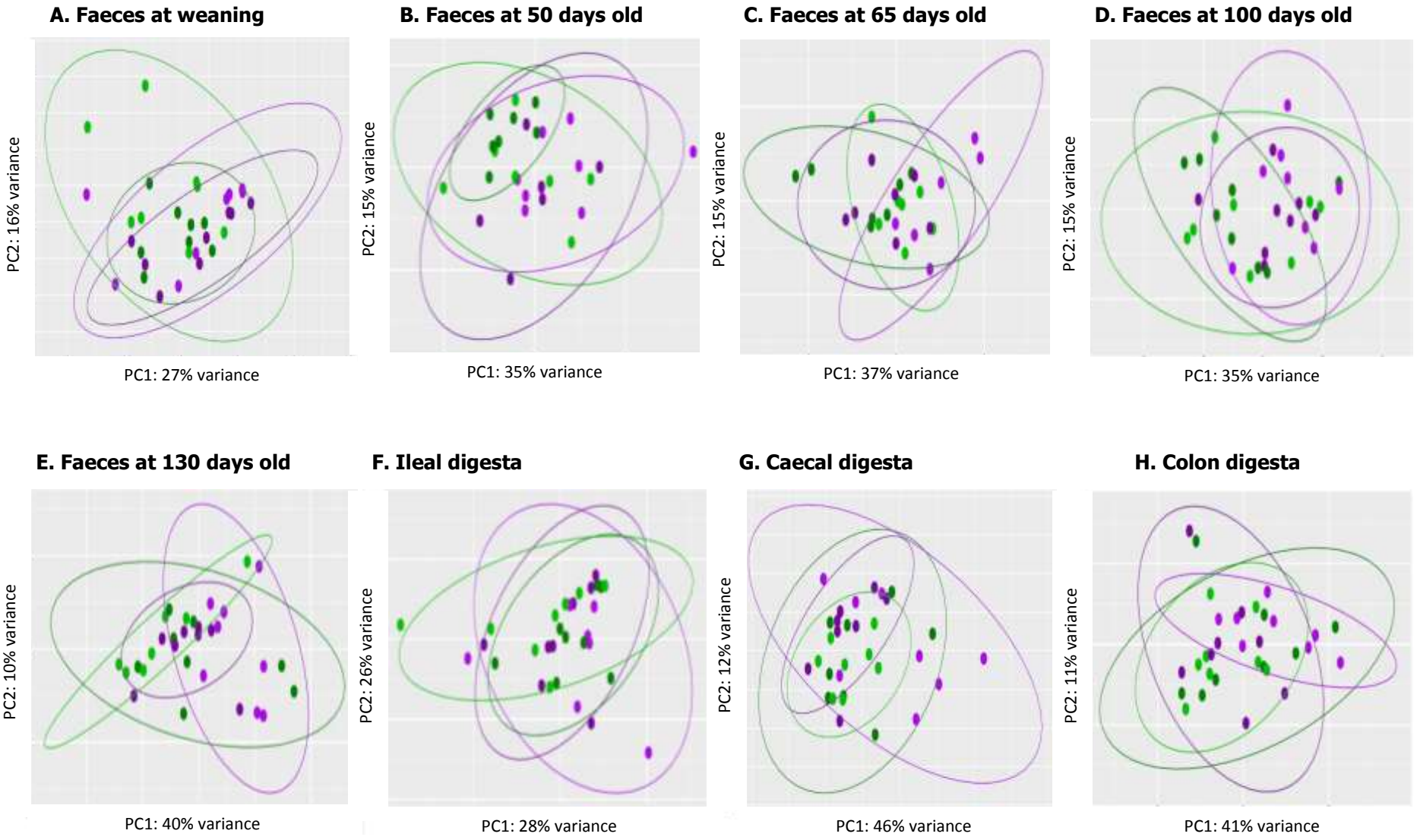

A. Faeces at weaning
PC2: 16% variance
PC1: 27% variance
B. Faeces at 50 days old
PC2: 15% variance
PC1: 35% variance
C. Faeces at 65 days old
PC2: 15% variance
PC1: 37% variance
D. Faeces at 100 days old
PC2: 15% variance
PC1: 35% variance
E. Faeces at 130 days old
PC2: 10% variance
PC1: 40% variance
F. Ileal digesta
PC2: 26% variance
PC1: 28% variance
G. Caecal digesta
PC2: 12% variance
PC1: 46% variance
H. Colon digesta
PC2: 11% variance
PC1: 41% variance
Control sow
Control
Inulin
FMT sow
Control
Inulin

Figure S5.2. Effect of faecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on Principal Component Analysis (PCA) plot (at the genus level) according to overall treatment and sample type

32 pigs selected; Sow treatment level: Control, n=16; FMT, n=16; Pig treatment level: Control, n=16; Inulin, n=16. The amount of variance is depicted by the percentages on each axis.

6. Overall conclusions

- Ranking pigs on divergence in RFI was useful, as it allowed the selection of animals that had similar growth rates with less feed consumed. However, as RFI is a regression equation that takes into account gender, body back fat and muscle, calculations can be time consuming and laborious

- Porcine intestinal microbial composition and predicted functionality was linked with FE, albeit it was mainly taxa found at quite low relative abundance that were FE-associated. However, these FE-associated taxa varied with geographical location, even when genetics, management, diet etc. were controlled. This indicates the rearing environment influences the intestinal microbiota

- Microbes involved in nutrient digestion and host health were enriched in low RFI (more feed efficient) pigs. These microbes could be used as biomarkers for feed efficiency, or fed as probiotics. However, advancements in culturing techniques are needed, and due to the influence of rearing environment, site-specific approaches may be necessary, although this is an issue from a commercial point of view

- FMT (using an inoculum derived from highly feed efficient pigs) in sows and/or offspring resulted in reduced offspring body weight at slaughter, indicating that FMT was not a useful strategy to improve FE in pigs

- FMT in sows and/or offspring resulted in a reduction in the abundance of microbes known to play a role in nutrient digestion and gut health, together with an increase in bacteria potentially pathogenic to pigs. Furthermore, a reduced

villus height and area, as well as an increased number of goblet cells present along the villi were observed due to FMT, indicating poorer absorptive capabilities, possibly explaining the lower body weight at slaughter

- The addition of inulin to the diet of offspring born to FMT-treated sows showed an improvement in RFI, indicating a beneficial combinative effect of inulin and FMT. Inulin may have improved the microbes implanted in sows due to FMT. However, no other improvements in growth were observed, and FMT resulted in a reduced body weight at slaughter in offspring. Beneficial modulation of the intestinal microbiota was observed, albeit for bacterial taxa at low relative abundance. Other prebiotics may prove more useful for improving overall growth and FE in pigs, and may be beneficial in promoting the proliferation of the microbiota established due to FMT

7. Overall discussion

The objectives of this work were firstly to rank pigs on divergence in RFI, a metric of FE, and to investigate the intestinal microbiota and associated physiological parameters of low versus high RFI pigs. Following this, manipulation of the intestinal microbiota in order to improve FE was investigated, using either FMT in sows and/or offspring, or FMT in sows with/without the supplementation of a prebiotic, inulin, to the diet of offspring.

As FE is critical in pig production, there is a constant need to investigate strategies to improve FE, in order to reduce production costs as well as environmental emissions from pigs. The intestinal microbiome is a complex ecosystem of microbes, mainly bacteria, located along the GIT, and interest has increased in recent years, as it is now considered an important organ in itself (Mach et al., 2015, Buzoianu et al., 2013, Metzler-Zebeli et al., 2016, Kim and Isaacson, 2015a). Bacteria present in the pig GIT are well known to play a role in nutrient digestion, metabolism and development and regulation of the immune response (Fouhse et al., 2016, Ramayo-Caldas et al., 2016). Therefore, there is the potential that the intestinal microbiome is linked with FE in pigs. Research has found that the intestinal microbiota is linked with growth rate (Ramayo-Caldas et al., 2016), where increased relative abundance of *Prevotella*, a member of *Bacteroidetes*, was associated with higher body weight and better average daily gain in pigs. However, only one study to date has looked at bacterial populations that vary between good and poorly feed efficient pigs (Vigors et al., 2016a), and none have used high throughput sequencing of the 16S rRNA gene to identify microbial taxa potentially associated with FE.

In Chapters 2 and 3 of this thesis, a number of RFI-associated differences in microbial composition and potential functionality were found. Although many of the microbes found to differ between good and poorly feed efficient pigs were at low relative abundance (<2%), these may nonetheless be important for FE as it is the complex interplay between all of the micro-organisms present in the GIT that influence host health, nutrient digestion and absorption. These bacterial taxa may be targeted in the future to improve FE, used as probiotics to improve FE, or indeed used as biomarkers in order to identify highly feed efficient animals that could be incorporated in the breeding program in the future, if causality is established.

In the low RFI pigs (those with better FE) from Chapter 3, there was a higher relative abundance of the bacterial phylum *Lentisphaerae*, and interestingly, this was higher in abundance in the faeces collected from pigs in Chapter 4 at weaning, who received the faecal transplant once, which was derived from pigs in Chapter 3 with the lowest RFI. This suggests a possible benefit as regards the use of FMT in pigs, although results from Chapter 4 show the opposite. *Lentisphaerae* has been associated with improved FE in cattle, where a higher abundance was linked with a better body weight gain (Myer et al., 2015) so may be a useful to target in the future, although more work is needed to identify specific beneficial populations within this phylum.

Bacterial taxa that appeared in all experimental chapters, as either RFI-associated, or impacted by FMT or inulin were mostly members of *Firmicutes*. Members of this phylum play a key role in nutrient digestion (e.g. *Blautia, Cellulosilyticum, Ruminococcus*) and the production of VFAs (e.g. *Butyricicoccus, Faecalibacterium, Oribacterium*), mainly butyric acid, which is well known to be an energy source for

both the pig and the cells of the epithelial lining (Liu, 2015). As these bacterial taxa were the ones most impacted throughout the experimental chapters of this thesis, this may indicate that these may have a role in driving FE, and could be useful bacteria to target/manipulate in the future. Furthermore, a common microbe that was higher in abundance in low RFI pigs in Chapter 3 was *Ruminococcaceae* and at the genus level, *Ruminococcus* was higher in abundance in both sow faeces post-FMT treatment, and in offspring assigned to the FMT4 treatment group, at 50 days of age, in Chapter 4. These taxa play a role in nutrient digestion, as they are known to break down cellulose, otherwise indigestible to the host (Umu et al., 2015). Although these benefits were not observed in the pigs from Chapter 4, these taxa may still have an important role to play in porcine FE, and could potentially be targeted in the future or used as a probiotic.

In Chapter 3 common management practices were employed across all geographical locations, and variation in genetics, diet and housing were minimised, in order to see if there was an associated between the intestinal microbiota and FE in pigs. The major finding was that rearing environment, as indicated by geographical location, was much more influential on bacterial composition and predicted functionality, than FE. Similarly, work in humans has found that there is a distinct separation of the intestinal microbiome due to country of origin (Yatsunenko et al., 2012). However, some bacterial taxa associated with low RFI were common to two geographical locations, and these included microbes known to play a role in nutrient digestion and host health e.g. *Mucispirillum* and *Methanobrevibacter*, although these were at quite low relative abundance. Other microbes common across rearing environments that were associated with low RFI were uncultured members of *Bacteroidetes* and *Cyanobacteria*, which may potentially be cultured in the future. Further investigation into the potential use of

these bacterial taxa as biomarkers for FE, or as probiotics in order to improve FE may be warranted. However, given the limited number of microbes associated with RFI that were common across rearing environments (none were common across the four batches), intervention strategies may need to be performed on a site-specific basis, which is of course an issue from a commercial, and economic point of view.

The use of FMT has become quite a popular for the treatment of gastrointestinal diseases in humans, mainly *Clostridium difficile* infection, as it aims to re-populate the intestine with a more beneficial bacterial population (Borody and Khoruts, 2012). One of the main benefits of using FMT is that it supplies a full spectrum of bacteria, and may therefore be useful in improving FE in pigs. In chapter 4, FMT was used to alter the microbial profile of sows and/or their offspring, with the hope of improving FE. The faecal inoculum was prepared using faecal extracts from highly feed efficient pigs; those with the lowest RFI from ROI2 pigs in Chapter 3. The aim was to improve FE by seeding the gut with bacteria from highly feed efficient pigs, given that FE-associated microbes were identified in Chapter 2 and 3.

Pregnant sows received the FMT in late gestation, following a 7-day course of antibiotics to clear the digestive tract of any resident microbiota. The antibiotic treatment had a very strong influence on the microbiome, reducing bacterial diversity and total bacterial load, as expected, and altering bacterial composition at the genus level also. However, the impact of FMT on the faecal and colostrum microbiota of sows was minimal, but it appeared to restore the faecal microbiota back to its 'original' state (pre-antibiotic treatment), and so this could indicate that FMT may be a useful way to re-populate the porcine intestine following antibiotic treatment. Maternal influence

on the intestinal microbiota was very clear from this chapter, as a number of differences within the offspring microbiome were due to FMT in sows. This highlights the potential to use microbial modulation strategies in the sow to influence offspring microbiota, and potentially FE. As previous work has found that probiotics/prebiotics fed to the sow can influence subsequent offspring (Paßlack et al., 2015, Leonard et al., 2010), this may be a key area to target in the future.

It is well known that the porcine GIT is essentially sterile at birth, and so this is a key time to perform intervention studies. However, a surprising result was that the FMT, either in sows or in their offspring, led to a reduction in offspring body weight at ~155 days of age (slaughter). When examining the intestinal microbiome, FMT increased bacterial diversity in 50 day old pigs, which may be seen as a beneficial result, but this was not a consistent change throughout the lifetime. Furthermore, a reduction in abundance of bacterial populations (at the phylum and genus level) related to nutrient digestion and health was observed, and potential pathogens were increased in offspring faeces and in the intestinal digesta collected at slaughter. The negative impact of FMT on pig growth may indicate in utero effects due to FMT in sows, as well as altering the faecal and colostrum bacterial profile of the sows, direct transfer of microbes from the sow to the offspring may have occurred. Furthermore, it is possible that "undesirable" microbiota were present in the faecal inoculum, and this may have caused the depression in offspring body weight observed. This work suggests that FMT is not an ideal approach to improve FE in pigs, and more suitable faecal inoculum or a more targeted approach such as the use of probiotics, may be a better way to beneficially manipulate the microbiota. Future work on identifying a more suitable inoculum for FMT in pigs is needed, including optimum donor age, and timing of intervention in

order to fully elucidate if there is indeed a place for FMT as a manipulative tool to improve FE in pigs.

Chapter 5 investigated the influence of nutritional intervention on the intestinal microbiota composition and potential functionality with the view to improving FE. Here, the prebiotic inulin was added to the diet of weaner pigs for 6 weeks, from the sows administered FMT/not in Chapter 4. Dietary supplementation with inulin was performed with the aim of promoting the microbiota implanted in pigs due to FMT in sows. As conflicting results have been published regarding the benefits of inulin on pig growth, we hypothesised that the combination of FMT in sows and dietary inulin treatment may have an additive effect. However, inulin alone proved ineffective in terms of growth, as no improvements in growth rate or FE were observed, although beneficial microbes (e.g. *Prevotella, Bifidobacterium, Bacteroides*) and butyrate producers (*Faecalibacterium, Butyricimonas*) were present at a higher relative abundance. A reduction in bacterial genera containing some species which are pathogenic to pigs (e.g. *Streptococcus* and *Campylobacter)*, indicates beneficial modulation of the intestinal microbiota in pigs fed inulin. However, the overall aim of this thesis, and indeed the work conducted in Chapter 5, was to improve growth and FE, and as inulin did not directly achieve this, other prebiotics may be more advantageous for pig producers.

Microbial composition is key to understanding the intestinal microbiome, but bacterial diversity may be just as important. The amount of bacterial species present and how similar/dissimilar the bacterial members are can be a good indicator of health status and viability and survivability of each microbe present in the GIT (Ursell et al., 2012).

Although a higher α-diversity (number of species present in a sample) was observed in low RFI pigs (ROI2 only) in Chapter 3, this was not a common finding throughout the study, or across rearing environments, and likewise in Chapters 4 and 5, transient higher bacterial diversity was observed, which may explain the depression in offspring body weight observed in the latter 2. In humans, a higher bacterial diversity is deemed beneficial for intestinal health (Le Chatelier et al., 2013). Similarly, increased intestinal microbial diversity has recently been associated with reduced susceptibility to post-weaning diarrhoea in piglets (Dou et al., 2017), which is hugely desirable, given the issues pig producers face with poor growth around weaning Furthermore, ß-diversity, can show how similar or different samples are to each other. This is a huge benefit to understanding the intestinal microbiome, as faeces is commonly used as an indicator of microbiota present in the intestine, and from the ß-diversity plots in the experimental chapters of this thesis, it is clear that there is a huge difference between the faeces collected throughout the lifetime and the caecum, compared to the ileal digesta. This work indicates that further studies may be needed to get a clearer picture of the microbiome in the upper GIT, rather than relying on faeces. In Chapter 4 and 5, digesta was collected from the stomach and the jejunum, with the aim of investigating the microbiota present, but this failed due to low quantity DNA in the stomach, and degradation of DNA in the jejunum. Although successful sequencing of the microbiota in the upper GIT, we can get a better understanding of the link between the intestinal microbiome and FE.

Although investigating the microbial composition of the intestinal microbiome is very important, the functionality of the bacteria present may be even more so. Shotgun sequencing is an expensive method of analysis, so in this thesis we used the software

program PICRUSt to predict the functionality of the microbiota present. This proved to be useful, but can only be used as a guideline to estimate the function of the bacteria present, as the database was set up using data from the human microbiome project. There were no common FE-associated pathways identified across experimental chapters. However, a number of pathways involved in metabolism were altered, including biosynthesis of fatty acids, which were higher in abundance in low RFI pigs in the two batches from ROI in Chapter 3, and bile acid biosynthesis which was enhanced due to FMT treatment in Chapter 4 and the dietary inulin supplementation in Chapter 5. This may warrant future investigation also, as if metabolism pathways can be altered, a faster growing and more lean (desirable) pig can be produced.

Another way to measure functionality of the intestinal microbiome is to look at bacterial metabolites produced by bacterial fermentation. In terms of VFA production, due to microbial fermentation, only ileal isobutyric acid differed between high and low RFI pigs in Chapter 2, with more feed efficient pigs having higher concentrations. However, in Chapter 4, FMT in sows resulted in a reduced concentration of isobutyric acid in the colon of their offspring, and low RFI pigs in Chapter 3 had a lower concentration of butyric acid in the faeces collected just prior to slaughter, which is indicative of a better absorptive capability in the colon (Fernandes et al., 2014). Furthermore, FMT-treated pigs from Chapter 4, had higher ileal butyric acid concentration, but in Chapter 5, due to FMT in sows, or the supplementation of inulin to the diet of offspring, butyric acid was reduced in the caecum. This may suggest improved or altered nutrient digestion, or indeed better absorptive capability in these pigs. However, this was not reflected in the growth and FE of these pigs.

In terms of gut structure, which is indicative of health and absorptive capacity of the intestine, and indeed the microbial populations present in the GIT, the number of goblet cells in the ileum was lower in the low RFI pigs in Chapter 2. This may indicate a healthier and more efficient gut, given that goblet cells produce mucin, which acts as a physical barrier to pathogenic invasion, but it can also inhibit nutrient absorption across the intestine (Montagne et al., 2004). In contrast to this, pigs from Chapter 4 had a higher number of goblet cells due to FMT in either sows or offspring, and this may indicate potential infection (pathogenic bacteria adhering to the intestinal wall) or indeed an over-stimulated intestinal barrier, due to the "foreign" microbes implanted due to FMT, and therefore reduced nutrient absorption across the intestinal wall. Furthermore, although villus height and crypt depth did not differ between RFI-ranked pigs in Chapter 2, there was a clear effect of FMT on villus structure in Chapters 4 and 5; due to FMT in sows, pigs had reduced villus height and area in the ileum, which may explain the reduced body weight at slaughter observed in pigs from both Chapters 4 and 5, as it is well known that longer villi and shorter crypts are better for nutrient absorption (Lalles, 2007), and therefore enhanced growth and FE.

Overall, the work in this thesis has provided one of the first set of potential microbial biomarkers for FE in pigs, and has identified RFI-associated microbes that were common across two geographical locations, that could be investigated as potential probiotics and/or targeted by dietary means or FMT in order to improve FE. Although the use of FMT in sows and their offspring was not a successful approach to beneficially manipulate the microbiota to improve FE, results from this work demonstrate the major influence of both maternal and early life intestinal microbiota on lifetime pig performance. The supplementation of inulin, to the diet of pigs (born to

control or FMT-treated sows) did not improve growth, but did beneficially alter bacterial populations in the intestine. However, other prebiotics may have a more suitable for the improvement of porcine FE. In combination with FMT, prebiotics may prove beneficial in maintaining/promoting the bacterial populations established, throughout the lifetime.

Future work would include: (1) cause and effect studies, to establish if it is the microbiota that influences FE, or if it is FE that influences the microbiota; (2) targeted approaches to manipulate the intestinal microbiota such as probiotics; (3) the use of FMT in pigs as a tool to improve FE; optimum age of donor/recipient, timing of intervention, route of entry (4) alternative feed additives (prebiotic or otherwise) that may beneficially modulate the intestinal microbiota, but also improve FE in pigs; (5) shotgun sequencing to investigate functionality of the microbiome in FE-ranked pigs, in order to fully understand the role of the intestinal microbiome in determining/influencing FE in pigs.

8. Bibliography

Alang, N. & Kelly, C. R. 2015. Weight gain after fecal microbiota transplantation. *OFID.,* 2**,** 1-2.

Aliprantis, A. O., Yang, R. B., Mark, M. R., Suggett, S., Devaux, B., Radolf, J. D., Klimpel, G. R., Godowski, P. & Zychlinsky, A. 1999. Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science,* 285**,** 736-739.

Awad, W. A., Ghareeb, K., Paßlack, N. & Zentek, J. 2013. Dietary inulin alters the intestinal absorptive and barrier function of piglet intestine after weaning. *Res. Vet. Sci.,* 95**,** 249-254.

Ballard, S. T., Hunter, J. H. & Taylor, A. E. 1995. Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. *Annu. Rev. Nutr.,* 15**,** 35-55.

Belzer, C., Gerber, G. K., Roeselers, G., Delaney, M., Dubois, A., Liu, Q., Belavusava, V., Yeliseyev, V., Houseman, A., Onderdonk, A., Cavanaugh, C. & Bry, L. 2014. Dynamics of the microbiota in response to host infection. *PLoS ONE,* 9**,** 1-17.

Bendixen, E., Danielsen, M., Hollung, K., Gianazza, E. & Miller, I. 2011. Farm animal proteomics—a review. *J. Proteomics,* 74**,** 282-293.

Bereskin, B. 1986. A genetic analysis of feed conversion efficiency and associated traits in swine. *J. Anim. Sci.,* 62**,** 910-917.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract of various species. *Physiol. Rev.,* 70**,** 567-590.

Bergstrom, J. R., Tokach, M. D., Dritz, S. S., Nelssen, J. L., Derouchey, J. M. & Goodband, R. D. 2008. Effects of feeder design on growth performance and carcass characterisics of finishing pigs. *Swine Day,* 2008**,** 196-203.

Berni Canani, R., Sangwan, N., Stefka, A. T., Nocerino, R., Paparo, L., Aitoro, R., Calignano, A., Khan, A. A., Gilbert, J. A. & Nagler, C. R. 2016. *Lactobacillus rhamnosus* GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *ISME J.,* 10**,** 742-750.

Berry, D. P. & Crowley, J. J. 2012. Residual intake and body weight gain: A new meausre of feed efficiency in growing cattle. *J. Anim. Sci.,* 90**,** 109-115.

Bikker, P., Dirkzwager, A., Fledderus, J., Trevisi, P., Le Huerou-Luron, I., Lalles, J. P. & Awati, A. 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly-weaned piglets. *J. Anim. Sci.,* 84**,** 3337-3345.

Blanton, L. V., Charbonneau, M. R., Salih, T., Barratt, M. J., Venkatesh, S., Ilkaveya, O., Subramanian, S., Manary, M. J., Trehan, I., Jorgensen, J. M., Fan, Y.-M., Henrissat, B., Leyn, S. A., Rodionov, D. A., Osterman, A. L., Maleta, K. M., Newgard, C. B., Ashorn, P., Dewey, K. G. & Gordon, J. I. 2016. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science,* 351**,** 33111-33117.

Bolger, A. M., Lohse, M. & Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics,* 30**,** 2114-2120.

Borody, T., Brandt, L. J. & Paramsothy, S. 2014. Therapeutic faecal microbiota transplantation: Current status and future developments. *Curr. Opin. Gastroenterol.,* 30**,** 97-105.

Borody, T. & Khoruts, A. 2012. Faecal microbiota transplantation and emerging applications. *A. Nat. Rev. Gastroenterol. Hepatol.,* 9**,** 88-96.

Burger-Van Paassen, N., Vincent, A., Puiman, P. J., Van Der Sluis, M., Bouma, J., Boehm, G., Van Goudoever, J. B., Van Seuningen, I. & Renes, I. B. 2009. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: Implications for epithelial protection. *Biochem. J.,* 420**,** 211-219.

Buzoianu, S. G., Walsh, M. C., Rea, M. C., O'sullivan, O., Cotter, P. D., Ross, R. P., Gardiner, G. E. & Lawlor, P. G. 2012a. High-throughput sequence-based analysis of the intestinal microbiota of weanling pigs fed genetically modified MON810 maize expressing

Bacillus thuringiensis Cry1Ab (Bt Maize) for 31 Days. *Appl. Environ. Microbiol.,* 78**,** 4217-4224.

Buzoianu, S. G., Walsh, M. C., Rea, M. C., O'sullivan, O., Crispie, F., Cotter, P. D., Ross, R. P., Gardiner, G. E. & Lawlor, P. G. 2012b. The effect of feeding Bt MON810 maize to pigs for 110 days on intestinal microbiota. *PLoS ONE,* 7**,** 1-9.

Buzoianu, S. G., Walsh, M. C., Rea, M. C., Quigley, L., O'sullivan, O., Cotter, P. D., Ross, R. P., Gardiner, G. E. & Lawlor, P. G. 2013. Sequence-based analysis of the intestinal microbiota of sows and their offspring fed genetically modified maize expressing a truncated form of *Bacillus thuringiensis* Cry1ab protein (Bt maize). *Appl. Environ. Microbiol.,* 79**,** 7735-7744.

Cai, W., Casey, D. & Dekkers, J. 2008. Selection response and genetic parameters for residual feed intake in Yorkshire swine. *J. Anim. Sci.,* 86**,** 287-298.

Calder, P. C. 2001. Polyunsaturated fatty acids, inflammation, and immunity. *Lipids,* 36**,** 1007-1024.

Canibe, N. & Jensen, B. B. 2003. Fermented and non fermented liquid feed to growing pigs: effects on aspects of gastrointestinal ecology and growth performance. *J. Anim. Sci.,* 81**,** 2019-2031.

Carberry, C. A., Waters, S. M., Kenny, D. A. & Creevey, C. J. 2014. Rumen methanogenic genotypes differ in abundance according to host residual feed intake phenotype and diet type. *Appl. Environ. Microbiol.,* 80**,** 2039-2039.

Casey, P. G., Gardiner, G. E., Casey, G., Bradshaw, B., Lawlor, P. G., Lynch, P. B., Leonard, F. C., Stanton, C., Ross, R. P., Fitzgerald, G. F. & Hill, C. 2007. A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with *Salmonella enterica* Serovar Typhimurium. *Appl. Environ. Microbiol.,* 73**,** 1858-1863.

Chattopadhyay, M. K. 2014. Use of antibiotics as feed additives: A burning question. *Front. Microbiol.,* 5**,** 1-7.

Chaucheyras-Durand, F. & Durand, H. 2010. Probiotics in animal nutrition and health. *Benef. Microbes,* 1**,** 3-9.

Chen, H., Mao, X., He, J., Yu, B., Huang, Z., Yu, J., Zheng, P. & Chen, D. 2013. Dietary fibre affects intestinal mucosal barrier function and regulates intestinal bacteria in weaning piglets. *Br. J. Nutr.,* 110**,** 1837-1848.

Chen, H., Mao, X. B., Che, L. Q., Yu, B., He, J., Yu, J., Han, G. Q., Huang, Z. Q., Zheng, P. & Chen, D. W. 2014. Impact of fiber types on gut microbiota, gut environment and gut function in fattening pigs. *Anim. Feed Sci. Technol.,* 195**,** 101-111.

Chen, J., Tellez, G., Richards, J. D. & Escobar, J. 2015. Identification of potential biomarkers for gut barrier failure in broiler chickens. *Front. Vet. Sci.,* 2**,** 1-10.

Chen, P., Baas, T.J., Mabry, J.W., Dekkers, J.C.M., and Koehler, K.J. 2002. Genetic parameters and trends for lean growth rate and its components in U.S. Yorkshire, Duroc, Hampshire and Landrace pigs. *J. Anim. Sci.,* 80**,** 2062-2070.

Choct, M., Selby, E.A.D., Cadogan, D.J., and Campbell, R.G. 2004. Effects of particle size, processing, and dry or liquid feeding on performance of pigs. *Aust. J. Agric. Res.,* 55**,** 237-245.

Clarke, S. F., Murphy, E. F., O'sullivan, O., Lucey, A. J., Humphreys, M., Hogan, A., Hayes, P., O'reilly, M., Jeffery, I. B., Wood-Martin, R., Kerins, D. M., Quigley, E., Ross, R. P., O'toole, P. W., Molloy, M. G., Falvey, E., Shanahan, F. & Cotter, P. D. 2014. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut,* 63**,** 1913-1920.

Collins, C. L., Philpotts, A.C., and Henman, D.J. 2009. Improving growth performance of finishing pigs with high fat diets. *Anim. Prod. Sci.,* 49**,** 262-267.

Collins, S. M., Kassam, Z. & Bercik, P. 2013. The adoptive transfer of behavioral phenotype via the intestinal microbiota: experimental evidence and clinical implications. *Curr. Opin. Microbiol.,* 16**,** 240-245.

Cox, J., And Mann, M. 2007. Is proteomics the new genomics. *Cell,* 130**,** 395-398.

Cushing, K., Alvarado, D. M. & Ciorba, M. A. 2015. Butyrate and mucosal inflammation: New scientific evidence supports clinical observation. *Clin. Trans. Gastroenterol.,* 6**,** 1-2.

Danbred. 2017. *DanBred International-Swine genetics* [Online]. Available: http://www.danbredint.dk/home.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J. & Turnbaugh, P. J. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature,* 505**,** 559-563.

Davidson, M. H. & Maki, K. C. 1999. Effects of dietary inulin on serum lipids. *J. Nutr.,* 129**,** 1474s-1477s.

De Lange, C. F. M., Pluske, J., Gong, J. & Nyachoti, C. M. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. *Livest. Sci.,* 134**,** 124-134.

De Vos, W. M. 2013. Fame and future of faecal transplantations- developing next-generation therapies with synthetic microbiomes. *Microb. Biotechnol.,* 6**,** 316-325.

Deplancke, B. & Gaskins, H. R. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am. J. Clin. Nutr.,* 73**,** 1131-1141.

Deutscher, J., Francke, C. & Postma, P. W. 2006. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. *Microbiol. Mol. Biol. Rev.,* 70**,** 939-1031.

Diao, H., Yan, H. L., Xiao, Y., Yu, B., Yu, J., He, J., Zheng, P., Zeng, B. H., Wei, H., Mao, X. B. & Chen, D. W. 2016. Intestinal microbiota could transfer host gut characteristics from pigs to mice. *BMC Microbiol.,* 16**,** 1-16.

Dou, S., Gadonna-Widehem, P., Rome, V., Hamoudi, D., Rhazi, L., Lakhal, L., Larcher, T., Bahi-Jaber, N., Pinon-Quintana, A., Guyonvarch, A., Huërou-Luron, I. L. E. & Abdennebi-Najar, L. 2017. Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. *PLoS ONE,* 12**,** 1-20.

Dung, N. N. X., Manh, L. H. & Ogle, B. 2005. Effects of fermented liquid feeds on the performance, digestibility, nitrogen retention and plasma urea nitrogen of growing finishing pigs. *LRRD.,* 17**,** 1-12.

Dunkelberger, J. R., Boddicker, N. J., Serão, N. V. L., Young, J. M., Rowland, R. R. R. & Dekkers, J. C. M. 2015. Response of pigs divergently selected for residual feed intake to experimental infection with the PRRS virus. *Livest. Sci.,* 177**,** 132-141.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics,* 26**,** 2460-2461.

Ellekilde, M., Selfjord, E., Larsen, C. S., Jakesevic, M., Rune, I., Tranberg, B., Vogensen, F. K., Nielsen, D. S., Bahl, M. I., Licht, T. R., Hansen, A. K. & Hansen, C. H. F. 2014. Transfer of gut microbiota from lean and obese mice to antibiotic-treated mice. *Sci. Rep.,* 4**,** 1-8.

Englund, S., Hård Af Segerstad, C., Arnlund, F., Westergren, E. & Jacobson, M. 2012. The occurrence of *Chlamydia* spp. in pigs with and without clinical disease. *BMC Vet. Res.,* 8**,** 1-6.

Fabian, J., Chiba, L.I., Frobish, L.T., Mcelhenney, W.H., Kuhlers, D.L., and Nadarajah, K. 2004. Compensatory growth and nitrogen balance in grower finisher pigs. *J. Anim. Sci.,* 82**,** 2579- 2587.

Fao/Who. 2001. *Health and nutritional properties of probiotics in food* [Online]. Available: ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf

Fernandes, J., Su, W., Rahat-Rozenbloom, S., Wolever, T. M. S. & Comelli, E. M. 2014. Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. *Nutr. Diab.,* 4**,** 1-7.

Fernandez, L., Langa, S., Martin, V., Maldonado, A., Jimenez, E., Martin, R. & Rodriguez, J. M. 2013. The human milk microbiota: origin and potential roles in health and disease. *Pharmacol. Res.,* 69**,** 1-10.

Ferrari, B., Winsley, T., Ji, M. & Neilan, B. 2014. Insights into the distribution and abundance of the ubiquitous *candidatus Saccharibacteria* phylum following tag pyrosequencing. *Sci. Rep.,* 4**,** 1-9.

Flint, H. J. & Bayer, E. A. 2008a. Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. *Ann. N. Y. Acad. Sci.,* 1125**,** 280-288.

Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R. & White, B. A. 2008b. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat. Rev. Microbiol.,* 6**,** 121-131.

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P. & Forano, E. 2012. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes,* 3**,** 289-306.

Fouhse, J. M., Zijlstra, R. T. & Willing, B. P. 2016. The role of gut microbiota in the health and disease of pigs. *Ani. Front.,* 6**,** 30-36.

Fouhy, F., Deane, J., Rea, M. C., O'sullivan, O., Ross, R. P., O'callaghan, G., Plant, B. J. & Stanton, C. 2015. The effects of freezing on faecal microbiota as determined using MiSeq sequencing and culture-based investigations. *PLoS ONE,* 10**,** e0119355.

Frantz, N., Nelssen, J. L., Derouchey, J. M., Goodband, R. D., Tokach, M. D. & Dritz, S. S. 2003. Effects of a prebiotic, inulin, and a direct fed microbial on growth performance of weanling pigs. *Swine Day,* 2003**,** 1-5.

Frese, S. A., Parker, K., Calvert, C. C. & Mills, D. A. 2015. Diet shapes the gut microbiome of pigs during nursing and weaning. *Microbiome,* 3**,** 1-10.

Fushimi, T., Suruga, K., Oshima, Y., Fukiharu, M., Tsukamoto, Y. & Goda, T. 2007. Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *Br. J. Nutr.,* 95**,** 916-924.

Garry, B. P., Fogarty, M., Curran, T. P., O'connell, M. J. & O'doherty, J. V. 2007. The effect of cereal type and enzyme addition on pig performance, intestinal microflora, and ammonia and odour emissions. *Animal,* 1**,** 751-757.

Gibson, G. R. & Roberfroid, M. B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.,* 125**,** 1401-1412.

Giles, T. A., Belkhiri, A., Barrow, P. A. & Foster, N. 2017. Molecular approaches to the diagnosis and monitoring of production diseases in pigs. *Res. Vet. Sci.,* 114**,** 266-272.

Gonyou, H. & Lou, Z. 2000. Effects of eating space and availability of water in feeders on productivity and eating behavior of grower/finisher pigs. *J. Anim. Sci.,* 78**,** 865-870.

Goodrich, Julia k., Waters, Jillian l., Poole, Angela c., Sutter, Jessica l., Koren, O., Blekhman, R., Beaumont, M., Van treuren, W., Knight, R., Bell, Jordana t., Spector, Timothy d., Clark, Andrew g. & Ley, Ruth e. 2014. Human genetics shape the gut microbiome. *Cell,* 159**,** 789-799.

Goto, Y. & Kiyono, H. 2012. Epithelial barrier: an interface for the cross-communication between gut flora and immune system. *Immunol. Rev.,* 245**,** 147-163.

Goyette-Desjardins, G., Auger, J.-P., Xu, J., Segura, M. & Gottschalk, M. 2014. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent-an update on the worldwide distribution based on serotyping and sequence typing. *Emerg. Microbes. Infect.,* 3**,** 1-20.

Grela, E. R., Kowalczyk-Pecka, D., Hanczakowska, E. & Matras, J. 2016. Effect of inulin and a probiotic supplement in the diet of pigs on selected traits of the gastrointestinal microbiome. *Med. Weter.,* 72**,** 448-452.

Grela, E. R., Sobolewska, S., Kowalczuk-Vasilev, E. & Krasucki, W. 2014. Effect of dietary inulin source on piglet performance, immunoglobulin concentration, and plasma lipid profile. *Bull. Vet. Inst. Pulawy.,* 58**,** 453-458.

Grubbs, J., Dekkers, J., Huff-Lonergan, E., Tuggle, C. & Lonergan, S. 2016. Identification of potential serum biomarkers to predict feed efficiency in young pigs. *J. Anim. Sci.,* 94**,** 1482-1492.

Guerin-Danan, A., C., and Szylit, O. 1999. Storage of intestinal bacteria in samples frozen with glycerol. *Microb. Ecol. Health Dis.,* 11**,** 180-182.

Halas, D., Hansen, C. F., Hampson, D. J., Kim, J.-C., Mullan, B. P., Wilson, R. H. & Pluske, J. R. 2010. Effects of benzoic acid and inulin on ammonia–nitrogen excretion, plasma urea levels, and the pH in faeces and urine of weaner pigs. *Livest. Sci.,* 134**,** 243-245.

Han, G. G., Lee, J.-Y., Jin, G.-D., Park, J., Choi, Y. H., Chae, B. J., Kim, E. B. & Choi, Y.-J. 2016. Evaluating the association between body weight and the intestinal microbiota of weaned piglets via 16S rRNA sequencing. *Vet. Microbiol.,* 196**,** 55-62.

Harris, D. L. 1970. Breeding for efficiency in livestock production: Defining the economic objectives. *J. Anim. Sci.,* 30**,** 860-865.

Heinritz, S. N., Weiss, E., Eklund, M., Aumiller, T., Louis, S., Rings, A., Messner, S., Camarinha-Silva, A., Seifert, J. & Bischoff, S. C. 2016. Intestinal microbiota and microbial metabolites are changed in a pig model fed a high-fat/low-fiber or a low-fat/high-fiber diet. *PLoS ONE,* 11**,** 1-21.

Herd, R. M., And Arthur, P.F. 2009. Physiological basis for residual feed intake. *J. Anim. Sci.,* 87**,** 64-71.

Hermes, R. G., Molist, F., Ywazaki, M., Nofrarias, M., Gomez De Segura, A., Gasa, J., and Perez, J.F. 2009. Effect of dietary level of protein and fiber on the productive performance and health status of piglets. *J. Anim. Sci.,* 87**,** 3569-3577.

Hermitage. 2017. *Genetics/AI* [Online]. Available: http://www.hermitagegenetics.ie/ai-pig-genetics/.

Hespell, R. B., Wolf, R. & Bothast, R. J. 1987. Fermentation of xylans by *Butyrivibrio fibrisolvens* and other ruminal bacteria. *Appl. Environ. Microbiol.,* 53**,** 2849-2853.

Hillmann, E., Schrader, L., Mayer, C. & Gygax, L. 2008. Effects of weight, temperature and behaviour on the circadian rhythm of salivary cortisol in growing pigs. *Animal,* 2**,** 405-409.

Hirayama, K. 1999. Ex-germfree mice harbouring intestinal microbiota derived from other animal species as an experimental model for ecology and metabolism of intestinal bacteria. *Exp. Anim.,* 48**,** 219-227.

Holck, J. T., Schinckel, A. P., Coleman, J. L., Wilt, V. M., Senn, M. K., Thacker, B. J., Thacker, E. L. & Grant, A. L. 1998. The influence of environment on the growth of commercial finisher pigs. *JSHAP.,* 6**,** 141-149.

Hou, C., Liu, H., Zhang, J., Zhang, S., Yang, F., Zeng, X., Thacker, P. A., Zhang, G. & Qiao, S. 2015. Intestinal microbiota succession and immunomodulatory consequences after introduction of *Lactobacillus reuteri* I5007 in neonatal piglets. *PLoS ONE,* 10**,** 1-17.

Huang, Q., Wei, Y., Lv, Y., Wang, Y. & Hu, T. 2015. Effect of dietary inulin supplements on growth performance and intestinal immunological parameters of broiler chickens. *Livest. Sci.,* 180**,** 172-176.

Hurst, D., Clarke, L. & Lean, I. J. 2008. Effect of liquid feeding at different water to feed ratios on the growth performance of growing finishing pigs. *Animal,* 2**,** 1297-1302.

Jackson, P. G. G. 2007. *Appendix 3 laboratory reference values: biochemistry*.

Jewell, K. A., Mccormick, C. A., Odt, C. L., Weimer, P. J. & Suen, G. 2015. Ruminal bacterial community composition in dairy cows is dynamic over the course of two lactations and correlates with feed efficiency. *Appl. Environ. Microbiol.,* 81**,** 4697-4710.

Jiang, W., Wu, N., Wang, X., Chi, Y., Zhang, Y., Qiu, X., Hu, Y., Li, J. & Liu, Y. 2015. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci. Rep.,* 5**,** 1-7.

Johnson, C., Kliebenstein, J., Zimmerman, J., and Ellis, S. 2008. Effect of feed cost on the economic impact of PRRS *Iowa State University Animal Industry Report*

Just, A. 1984. Nutritional manipulation and interpretation of body compositional differences in growing swine. *J. Anim. Sci.,* 58**,** 740-752.

Kaakoush, N. O. 2015. Insights into the role of *Erysipelotrichaceae* in the human host. *Front. Cell. Infect. Microbiol.,* 5**,** 1-4.

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayama, T., Kawashima, S., Okuda, S., Tokimatsu, T. & Yamanishi, Y. 2008. KEGG for linking genomes to life and the environment. *Nucleic Acids Res.,* 36**,** D480-484.

Karovicova, J. & Kohajdova, Z. 2003. Biogenic amines in food *Chem. Pap.,* 59**,** 70-79.

Kelly, C. R. 2015. Editorial: a simple faecal preparation protocol for faecal microbiota transplantation. *Aliment. Pharmacol. Ther.,* 41**,** 320-320.

Kelly, D. & Mulder, I. E. 2012. Microbiome and immunological interactions. *Nutr. Rev.,* 70 S18-30.

Kelly, G. 2008. Inulin-type prebiotics-a review: part 1. *Altern. Med. Rev.,* 13**,** 315-329.

Kennelly, J., Aherne, F. & Sauer, W. 1981. Volatile fatty acid production in the hindgut of swine. *Can. J. Anim. Sci.,* 61**,** 349-361.

Kien, C. L., Blauwiekel, R., Bunn, J. Y., Jetton, T. L., Frankel, W. L. & Holst, J. J. 2007. Cecal infusion of butyrate increases intestinal cell proliferation in piglets. *J. Nutr.,* 137**,** 916-922.

Kim, H. B., Borewicz, K., White, B. A., Singer, R. S., Sreevatsan, S., Tu, Z. J. & Isaacson, R. E. 2011. Longitudinal investigation of the age-related bacterial diversity in the feces of commercial pigs. *Vet. Microbiol.,* 153**,** 124-133.

Kim, H. B. & Isaacson, R. E. 2015a. The pig gut microbial diversity: Understanding the pig gut microbial ecology through the next generation high throughput sequencing. *Vet. Microbiol.,* 177**,** 242-251.

Kim, J., Nguyen, S. G., Guevarra, R. B., Lee, I. & Unno, T. 2015b. Analysis of swine fecal microbiota at various growth stages. *Arch. Microbiol.,* 197**,** 753-759.

Kim, Y., Koh, I. & Rho, M. 2015c. Deciphering the human microbiome using next-generation sequencing data and bioinformatics approaches. *Methods,* 79–80**,** 52-59.

Kim, Y. S. & Ho, S. B. 2010. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr. Gastroenterol. Rep.,* 12**,** 319-330.

King, G. 2006. *Animals and Environments* [Online]. University of Guelph: University of Guelph. Available: http://www.aps.uoguelph.ca/~gking/Ag_2350/animenv.htm.

Kingma, B., Frijns, A. & Van Marken, L. W. 2012. The thermoneutral zone: implications for metabolic studies. *Front. Biosci. (Elite Ed).* 4**,** 1975-1985.

Klem, T. B., Bleken, E., Morberg, H., Thoresen, S. I. & Framstad, T. 2010. Hematologic and biochemical reference intervals for Norwegian crossbreed grower pigs. *Vet. Clin. Pathol.,* 39**,** 221-226.

Koch, R. M., Swiger, L.A., Chamber, D., Gregory, K.E. 1963. Efficiency of feed use in beef cattle *J. Anim. Sci.,* 22**,** 486-494.

Komijn, R. E., Wisselink, H. J., Rijsman, V. M., Stockhofe-Zurwieden, N., Bakker, D., Van Zijderveld, F. G., Eger, T., Wagenaar, J. A., Putirulan, F. F. & Urlings, B. A. 2007. Granulomatous lesions in lymph nodes of slaughter pigs bacteriologically negative for *Mycobacterium avium* subsp. avium and positive for *Rhodococcus equi*. *Vet. Microbiol.,* 120**,** 352-357.

Konstantinov, S. R., Awati, A., Smidt, H., Williams, B. A., Akkermans, A. D. L. & De Vos, W. M. 2004. Specific response of a novel and abundant *Lactobacillus amylovorus*-like phylotype to dietary prebiotics in the guts of weaning piglets. *Appl. Environ. Microbiol.,* 70**,** 3821-3830.

Koropatkin, N. M., Cameron, E. A. & Martens, E. C. 2012. How glycan metabolism shapes the human gut microbiota. *Nat. Rev. Micro.,* 10**,** 323-335.

Kozłowska, I., Marć-Pieńkowska, J. & Bednarczyk, M. 2016. Beneficial aspects of inulin supplementation as a fructooligosaccharide prebiotic in monogastric animal nutrition–a review. *Ann. Anim. Sci.,* 16**,** 315-331.

Lalles, J. P., Bosi, P., Smidt, H., Stokes, C.R. 2007. Nutritional management of gut health in pigs around weaning. *Proc. Nutr. Soc.,* 66**,** 260-268.

Landgraf, S., Susenbeth, A., Knap, P. W., Looft, H., Plastow, G. S., Kalm, E. & Roehe, R. 2006. Developments of carcass cuts, organs, body tissues and chemical body composition during the growth of pigs. *Anim. Sci.,* 82**,** 889-899.

Langille, M. G. I., Zaneveld, J., Caporaso, J. G., Mcdonald, D., Knights, D., Reyes, J. A., Clemente, J. C., Burkepile, D. E., Vega Thurber, R. L., Knight, R., Beiko, R. G. & Huttenhower, C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotech.,* 31**,** 814-821.

Lara, G. H., Ribeiro, M. G., Leite, C. Q., Paes, A. C., Guazzelli, A., Da Silva, A. V., Santos, A. C. & Listoni, F. J. 2011. Occurrence of *Mycobacterium* spp. and other pathogens in lymph nodes of slaughtered swine and wild boars (*Sus scrofa*). *Res. Vet. Sci.,* 90**,** 185-188.

Lawlor, P., Lynch, P., Gardiner, G., Caffrey, P. & O'doherty, J. 2002. Effect of liquid feeding weaned pigs on growth performance to harvest. *J. Anim. Sci.,* 80**,** 1725-1735.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.-M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jorgensen, T., Brandslund, I., Nielsen, H. B., Juncker, A. S., Bertalan, M., Levenez, F., Pons, N., Rasmussen, S., Sunagawa, S., Tap, J., Tims, S., Zoetendal, E. G., Brunak, S., Clement, K., Dore, J., Kleerebezem, M., Kristiansen, K., Renault, P., Sicheritz-Ponten, T., De Vos, W. M., Zucker, J.-D., Raes, J., Hansen, T., Meta, H. I. T. C., Bork, P., Wang, J., Ehrlich, S. D. & Pedersen, O. 2013. Richness of human gut microbiome correlates with metabolic markers. *Nature,* 500**,** 541-546.

Lee, S. J., Shin, N. H., Ok, J. U., Jung, H. S., Chu, G. M., Kim, J. D., Kim, I. H. & Lee, S. S. 2009. Effects of dietary synbiotics from anaerobic microflora on growth performance, noxious gas emission and fecal pathogenic bacteria population in weaning pigs. *Asian-australas. J. Anim. Sci.,* 22**,** 1202-1208.

Lee, T., Clavel, T., Smirnov, K., Schmidt, A., Lagkouvardos, I., Walker, A., Lucio, M., Michalke, B., Schmitt-Kopplin, P., Fedorak, R. & Haller, D. 2017. Oral versus intravenous iron replacement therapy distinctly alters the gut microbiota and metabolome in patients with IBD. *Gut,* 66**,** 863-871.

Lefaucheur, L., Lebret, B., Ecolan, P., Louveau, I., Damon, M., Prunier, A., Billon, Y., Sellier, P. & Gilbert, H. 2011. Muscle characteristics and meat quality traits are affected by divergent selection on residual feed intake in pigs. *J. Anim. Sci.,* 89**,** 996-1010.

Leonard, S. G., Sweeney, T., Bahar, B., Lynch, B. P. & O'doherty, J. V. 2010. Effect of maternal fish oil and seaweed extract supplementation on colostrum and milk composition, humoral immune response, and performance of suckled piglets. *J. Anim. Sci.,* 88**,** 2988-2997.

Levine, U. Y., Looft, T., Allen, H. K. & Stanton, T. B. 2013. Butyrate-producing bacteria, including mucin degraders, from the swine intestinal tract. *Appl. Environ. Microbiol.,* 79**,** 3879-3881.

Levy, M., Blacher, E. & Elinav, E. 2016. Microbiome, metabolites and host immunity. *Curr. Opin. Microbiol.,* 35**,** 8-15.

Li, P., Piao, X., Ru, Y., Han, X., Xue, L. & Zhang, H. 2012. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. *Asian-australas. J. Anim. Sci.,* 25**,** 1617-1626.

Li, Q. & Patience, J. F. 2016. Factors involved in the regulation of feed and energy intake of pigs. *Anim. Feed Sci. Technol.,* 10**,** 1-12.

Lin, H., An, Y., Hao, F., Wang, Y. & Tang, H. 2016. Correlations of fecal metabonomic and microbiomic changes induced by high-fat diet in the pre-obesity state. *Sci. Rep.,* 6**,** 1-14.

Liu, H., Nguyen, Y. T., Nettleton, D., Dekkers, J. C. M. & Tuggle, C. K. 2016. Post-weaning blood transcriptomic differences between Yorkshire pigs divergently selected for residual feed intake. *BMC Genomics,* 17**,** 1-18.

Liu, Y. 2015. Fatty acids, inflammation and intestinal health in pigs. *J. Anim. Sci. Biotechnol.,* 6**,** 1-9.

Loh, G., Eberhard, M., Brunner, R. M., Hennig, U., Kuhla, S., Kleessen, B. & Metges, C. C. 2006. Inulin alters the intestinal microbiota and short-chain fatty acid concentrations in growing pigs regardless of their basal diet. *J. Nutr.,* 136**,** 1198-1202.

Loman, N. J., Constantinidou, C., Chan, J. Z. M., Halachev, M., Sergeant, M., Penn, C. W., Robinson, E. R. & Pallen, M. J. 2012. High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. *Nat. Rev. Micro.,* 10**,** 599-606.

Lopez, J., Jesse, G.W., Becker, B.A., and Ellersieck M.R. 1991. Effects of temperature on the performance of finishing swine: I. effects of a hot, diurnal temperature on average daily gain, feed intake and feed efficiency. *J. Anim. Sci.,* 69**,** 1842- 1849.

Louis, P. & Flint, H. J. 2017. Formation of propionate and butyrate by the human colonic microbiota. *Environ. Microbiol.,* 19**,** 29-41.

Love, M. I., Huber, W. & Anders, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.,* 15**,** 1-21.

Lynch, M. B., O'shea, C. J., Sweeney, T., Callan, J. J. & O'doherty, J. V. 2008. Effect of crude protein concentration and sugar-beet pulp on nutrient digestibility, nitrogen excretion, intestinal fermentation and manure ammonia and odour emissions from finisher pigs. *Animal,* 2**,** 425-434.

Lynch, M. B., Sweeney, T., Callan, B. F., J. J. & O'doherty, J. V. 2007. The effect of high and low dietary crude protein and inulin supplementation on nutrient digestibility, nitrogen excretion, intestinal microflora and manure ammonia emissions from finisher pigs. *Animal,* 1**,** 1112-1121.

Mach, N., Berri, M., Estellé, J., Levenez, F., Lemonnier, G., Denis, C., Leplat, J.-J., Chevaleyre, C., Billon, Y., Doré, J., Rogel-Gaillard, C. & Lepage, P. 2015. Early-life establishment of the swine gut microbiome and impact on host phenotypes. *Environ. Microbiol. Rep.,* 7**,** 554-569.

Magoc, T. & Salzberg, S. L. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics,* 27**,** 2957-2963.

Mani-López, E., García, H. S. & López-Malo, A. 2012. Organic acids as antimicrobials to control Salmonella in meat and poultry products. *Food Res. Int.,* 45**,** 713-721.

Mani, V., Harris, A. J., Keating, A. F., Weber, T. E., Dekkers, J. C. M. & Gabler, N. K. 2013. Intestinal integrity, endotoxin transport and detoxification in pigs divergently selected for residual feed intake. *J. Anim. Sci.,* 91**,** 2141-2150.

Mani, V., Weber, T. E., Baumgard, L. H. & Gabler, N. K. 2012. Growth and development symposium: endotoxin, inflammation, and intestinal function in livestock. *J. Anim. Sci.,* 90**,** 1452-1465.

Mao, S., Zhang, M., Liu, J. & Zhu, W. 2015. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. *Sci. Rep.,* 5**,** 1-14.

Maradiaga, N., Zeineldin, M., Aldridge, B. & Lowe, J. 2014. Influence of maternal microbial communities on the mucosal microbiome of neonatal pigs. *AASV,* 2014**,** 1-39.

Martens, E. C., Koropatkin, N. M., Smith, T. J. & Gordon, J. I. 2009. Complex glycan catabolism by the human gut microbiota: the *Bacteroidetes* sus-like paradigm. *J. Biol. Chem.,* 284**,** 24673-24677.

Martin, L., Cilieborg, M. S., Birck, M., Thymann, T. & Sangild, P. T. 2015. Fecal microbiota transplantation decreases necrotizing enterocolitis but is associated with increased neonatal mortality in preterm pigs. *jENS 2015.* Budapest.

Martin, L., Pieper, R., Schunter, N., Vahjen, W. & Zentek, J. 2013. Performance, organ zinc concentration, jejunal brush border membrane enzyme activities and mRNA expression in piglets fed with different levels of dietary zinc. *Arch. Anim. Nutr.,* 67**,** 248-261.

Mcmurdie, P. J. & Holmes, S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE,* 8**,** 1-11.

Mcphee, C. P. 1981. Selection for efficient growth in a pig herd. *Aust. J. Agric. Res.,* 32**,** 681-690.

Merrifield, C. A., Lewis, M. C., Berger, B., Cloarec, O., Heinzmann, S. S., Charton, F., Krause, L., Levin, N. S., Duncker, S., Mercenier, A., Holmes, E., Bailey, M. & Nicholson, J. K. 2016. Neonatal environment exerts a sustained influence on the development of the intestinal microbiota and metabolic phenotype. *ISME J.,* 10**,** 145-157.

Metzler-Zebeli, B. U., Eberspächer, E., Grüll, D., Kowalczyk, L., Molnar, T. & Zebeli, Q. 2015a. Enzymatically modified starch ameliorates postprandial serum triglycerides and lipid metabolome in growing pigs. *PLoS ONE,* 10**,** 1-19.

Metzler-Zebeli, B. U., Lawlor, P. G., Magowan, E., Mccormack, U. M., Curião, T., Hollmann, M., Ertl, R., Aschenbach, J. R. & Zebeli, Q. 2017a. Finishing pigs that are divergent in feed efficiency show small differences in intestinal functionality and structure. *PLoS ONE,* 12**,** 1-16.

Metzler-Zebeli, B. U., Lawlor, P. G., Magowan, E. & Zebeli, Q. 2016. Effect of freezing conditions on fecal bacterial composition in pigs. *Animals,* 6**,** 1-9.

Metzler-Zebeli, B. U., Mann, E., Schmitz-Esser, S., Wagner, M., Ritzmann, M. & Zebeli, Q. 2013. Changing dietary calcium-phosphorus level and cereal source selectively alters abundance of bacteria and metabolites in the upper gastrointestinal tracts of weaned pigs. *Appl. Environ. Microbiol.,* 79**,** 7264-7272.

Metzler-Zebeli, B. U., Schmitz-Esser, S., Mann, E., Grüll, D., Molnar, T. & Zebeli, Q. 2015b. Adaptation of the cecal bacterial microbiome of growing pigs in response to resistant starch type 4. *Appl. Environ. Microbiol.,* 81**,** 8489-8499.

Metzler-Zebeli, B. U., Trevisi, P., Prates, J. a. M., Tanghe, S., Bosi, P., Canibe, N., Montagne, L., Freire, J. & Zebeli, Q. 2017b. Assessing the effect of dietary inulin supplementation on gastrointestinal fermentation, digestibility and growth in pigs: A meta-analysis. *Anim. Feed Sci. Technol.,* 10**,** 1-13.

Miller, D. A., Suen, G., Bruce, D., Copeland, A., Cheng, J. F., Detter, C., Goodwin, L. A., Han, C. S., Hauser, L. J., Land, M. L., Lapidus, A., Lucas, S., Meincke, L., Pitluck, S., Tapia, R., Teshima, H., Woyke, T., Fox, B. G., Angert, E. R. & Currie, C. R. 2011. Complete genome sequence of the cellulose-degrading bacterium *Cellulosilyticum lentocellum*. *J. Bacteriol.,* 193**,** 2357-2358.

Millet, S., Kumar, S., De Boever, J., Ducatelle, R., De Brabander, D. 2012. Effect of feed processing on growth performance and gastric mucosa integrity in pigs from weaning to slaughter. *Anim. Feed Sci. Technol.,* 175**,** 175-181.

Million, M., Maraninchi, M., Henry, M., Armougom, F., Richet, H., Carrieri, P., Valero, R., Raccah, D., Vialettes, B. & Raoult, D. 2012. Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. *Int. J. Obes.,* 36**,** 817-825.

Missotten, J. A., Michiels, J., Degroote, J. & De Smet, S. 2015. Fermented liquid feed for pigs: an ancient technique for the future. *J. Ani. Sci. Biotech.,* 6**,** 1-9.

Montagne, L., Piel, C. & Lalles, J. P. 2004. Effect of diet on mucin kinetics and composition: nutrition and health implications. *Nutr. Rev.,* 62**,** 105-114.

Mpetile, Z. 2014. *Effect of divergent selection for residual feed intake on immune system of yorkshire pigs.* Master of Science, Iowa State University.

Mpetile, Z., Young, J. M., Gabler, N. K., Dekkers, J. C. M. & Tuggle, C. K. 2015. Assessing peripheral blood cell profile of Yorkshire pigs divergently selected for residual feed intake. *J. Anim. Sci.,* 93.

Mrode, R. A., And Kennedy, B.W. 1993. Genetic variation in measures of food efficiency in pigs and their genetic relationships with growth rate and backfat. *Anim. Prod.,* 56**,** 225-232.

Murtaugh, M. P., Baarsch, M. J., Zhou, Y., Scamurra, R. W. & Lin, G. 1996. Inflammatory cytokines in animal health and disease. *Vet. Immunol. Immunopathol.,* 54**,** 45-55.

Myer, P. R., Smith, T. P. L., Wells, J. E., Kuehn, L. A. & Freetly, H. C. 2015. Rumen microbiome from steers differing in feed efficiency. *PLoS ONE,* 10**,** 1-17.

Myers, A. J., Goodband, R.D., Tokach, M.D., Dritz, S.S., Derouchey, J.M., and Nelssen, J.L. 2013. The effects of diet form and feeder design on the growth performance of finishing pigs. *J. Anim. Sci.,* 91**,** 3420-3428.

Naidu, A., Bidlack, W. & Clemens, R. 1999. Probiotic spectra of lactic acid bacteria (LAB). *Crit. Rev. Food. Sci. Nutr.,* 39**,** 13-126.

Nieuwdorp, M. 2014. Faecal microbiota transplantation. *Br. J. Surg.,* 101**,** 887-888.

Niu, Q., Li, P., Hao, S., Zhang, Y., Kim, S. W., Li, H., Ma, X., Gao, S., He, L., Wu, W., Huang, X., Hua, J., Zhou, B. & Huang, R. 2015. Dynamic distribution of the gut microbiota and the relationship with apparent crude fiber digestibility and growth stages in pigs. *Sci. Rep.,* 5**,** 1-7.

Nofrarias, M., Manzanilla, E. G., Pujols, J., Gibert, X., Majo, N., Segales, J. & Gasa, J. 2006. Effects of spray-dried porcine plasma and plant extracts on intestinal morphology and on leukocyte cell subsets of weaned pigs. *J. Anim. Sci.,* 84**,** 2735-2742.

O'sullivan, L., Murphy, B., Mcloughlin, P., Duggan, P., Lawlor, P. G., Hughes, H. & Gardiner, G. E. 2010. Prebiotics from marine macroalgae for human and animal health applications. *Mar. Drugs,* 8**,** 2038-2064.

Pajarillo, E., Chae, J. P., Mp, B., Hb, K. & Kang, D. K. 2014. Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. *J. Gen. Appl. Microbiol.,* 60**,** 140-146.

Pajarillo, E. a. B., Chae, J. P., Balolong, M. P., Kim, H. B., Park, C.-S. & Kang, D.-K. 2015. Effects of probiotic *Enterococcus faecium* NCIMB 11181 administration on swine fecal microbiota diversity and composition using barcoded pyrosequencing. *Anim. Feed Sci. Technol.,* 201**,** 80-88.

Pamer, E. G. 2014. Fecal microbiota transplantation: effectiveness, complexities, and lingering concerns. *Mucosal. Immunol.,* 7**,** 210-214.

Pang, X., Hua, X., Yang, Q., Ding, D., Che, C., Cui, L., Jia, W., Bucheli, P., and Zhao, L. 2007. Inter-species transplantation of gut microbiota from humans to pigs. *ISME J.,* 1**,** 156-162.

Parra, S., Jaime, Agudelo, T., Jorge, Ortiz, L., Ramírez, M. C., Rodríguez, B. & López Herrera, A. 2011. Lipopolysaccharide (LPS) from *E. coli* has detrimental effects on the intestinal morphology of weaned pigs. *Rev. Colomb. Cienc. Pecu.,* 24**,** 585-597.

Partanen, K. H. a. Z., M. 1999. Organic acids for performance enhancement in pig diets. *Nutr. Res. Rev.,* 12**,** 117-145.

Paßlack, N., Vahjen, W. & Zentek, J. 2015. Dietary inulin affects the intestinal microbiota in sows and their suckling piglets. *BMC Vet. Res.,* 11**,** 1-8.

Patience, J. F., Rossoni-Serão, M. C. & Gutiérrez, N. A. 2015. A review of feed efficiency in swine: biology and application. *J. Anim. Sci. Biotechnol.,* 6**,** 1-9.

Patterson, J. K., Yasuda, K., Welch, R. M., Miller, D. D. & Lei, X. G. 2010. Supplemental dietary inulin of variable chain lengths alters intestinal bacterial populations in young pigs. *J. Nutr.,* 140**,** 2158-2161.

Pedersen, R., Andersen, A. D., Mølbak, L., Stagsted, J. & Boye, M. 2013. Changes in the gut microbiota of cloned and non-cloned control pigs during development of obesity: gut microbiota during development of obesity in cloned pigs. *BMC Microbiol.,* 13**,** 1-9.

Pedersen, R. M., Holt, H. M. & Justesen, U. S. 2011. *Solobacterium moorei* bacteremia: Identification, antimicrobial susceptibility, and clinical characteristics. *J. Clin. Microbiol.,* 49**,** 2766-2768.

Pic. 2017. *PIC-Pig Improvement Company* [Online]. Available: http://www.pic.com/.

Pié, S., Awati, A., Vida, S., Falluel, I., Williams, B. A. & Oswald, I. P. 2007. Effects of added fermentable carbohydrates in the diet on intestinal proinflammatory cytokine-specific mRNA content in weaning piglets1. *J. Anim. Sci.,* 85**,** 673-683.

Pierce, K., Callan, J., Mccarthy, P. & O'doherty, J. 2005. Performance of weanling pigs offered low or high lactose diets supplemented with avilamycin or inulin. *Anim. Sci.,* 80**,** 313-318.

Pimentel, M., Gunsalus, R. P., Rao, S. S. & Zhang, H. 2012. Methanogens in human health and disease. *Am. J. Gastroenterol. Suppl.,* 1**,** 28.

Pluske, J. R., Hampson, D. J. & Williams, I. H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: A review. *Livest. Prod. Sci.,* 51**,** 215-236.

Pluske, J. R., Pethick, D.W., Hopwood, D.E., and Hampson, D.J., 2002. Nutritional influences on some major enteric bacterial diseases of pigs. *Nutr. Res. Rev.,* 15**,** 333-371.

Pomar, C., Pomar, J., Dubeau, F., Joannopoulos, E., and Dussault, J.P. 2014. The impact of daily multiphase feeding on animal performance, body composition, nitrogen and phosphorus excretions, and feed costs in growing finishing pigs. *Animal,* 8**,** 704-713.

Pond, W. G. 2001. *Biology of the Domestic pig*

Poroyko, V., Hernandez, A.G., Alverdy, J.C., and Liu, D.C., 2012. Protocol for metatranscriptomic analysis of intestinal microbiota. *Nat. Proc.,* 10**,** 1-7.

Potter, M. L., Dritz, S.S., Tokach, M.D., Nelssen, J.L., Bergstrom, J.R., Goodband, R.D., and Derouchey, J.M. 2010. Effects of stocking density on lightweight pig performance prior to marketing. *Swine Day,* 2011**,** 262-265.

Pozuelo, M., Panda, S., Santiago, A., Mendez, S., Accarino, A., Santos, J., Guarner, F., Azpiroz, F. & Manichanh, C. 2015. Reduction of butyrate-and methane-producing microorganisms in patients with Irritable Bowel Syndrome. *Sci. Rep.,* 5**,** 1-12.

Prieto, M. L., O'sullivan, L., Tan, S. P., Mcloughlin, P., Hughes, H., O'donovan, O., Rea, M. C., Kent, R. M., Cassidy, J. P., Gardiner, G. E. & Lawlor, P. G. 2014. Evaluation of the efficacy and safety of a marine-derived *Bacillus* strain for use as an in-feed probiotic for newly weaned pigs. *PLoS ONE,* 9**,** 1-12.

R Team, R. D. C. 2010. *R: A language and environment for statistical computing* [Online].

Ramayo-Caldas, Y., Mach, N., Lepage, P., Levenez, F., Denis, C., Lemonnier, G., Leplat, J. J., Billon, Y., Berri, M., Dore, J., Rogel-Gaillard, C. & Estelle, J. 2016. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. *ISME J.,* 16**,** 1-5.

Ranjan, K. & Ranjan, N. 2013. Citrobacter: An emerging health care associated urinary pathogen. *Urol. Ann.,* 5**,** 313-314.

Reese, D., Liptrap, D., Parker, G., Cromwell, G. & Stahly, T. 1985. Factors affecting feed conversion in growing-finishing swine. *ASC-University of Kentucky, Cooperative Extension Service (USA).*

Riboulet-Bisson, E., Sturme, M. H. J., Jeffery, I. B., O'donnell, M. M., Neville, B. A., Forde, B. M., Claesson, M. J., Harris, H., Gardiner, G. E., Casey, P. G., Lawlor, P. G., O'toole, P. W. & Ross, R. P. 2012. Effect of *Lactobacillus salivarius* Bacteriocin Abp118 on the Mouse and Pig Intestinal Microbiota. *PLoS ONE,* 7**,** 1-12.

Richardson, E., Herd, R., Archer, J. & Arthur, P. 2004. Metabolic differences in Angus steers divergently selected for residual feed intake. *Anim. Prod. Sci.,* 44**,** 441-452.

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., Griffin, N. W., Lombard, V., Henrissat, B., Bain, J. R., Muehlbauer, M. J., Ilkayeva, O., Semenkovich, C. F., Funai, K., Hayashi, D. K., Lyle, B. J., Martini, M. C., Ursell, L. K., Clemente, J. C., Van Treuren, W., Walters, W. A., Knight, R., Newgard, C. B., Heath, A. C. & Gordon, J. I. 2013. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science,* 341**,** 1-12.

Rivera-Chavez, F., Zhang, L. F., Faber, F., Lopez, C. A., Byndloss, M. X., Olsan, E. E., Xu, G., Velazquez, E. M., Lebrilla, C. B., Winter, S. E. & Baumler, A. J. 2016. Depletion of butyrate-producing *Clostridia* from the gut microbiota drives an aerobic luminal expansion of *Salmonella*. *Cell Host Microbe,* 19**,** 443-454.

Roberfroid, M. 2007. Prebiotics: The Concept Revisited. *J. Nutr.,* 137**,** 830S-837S.

Rooks, M. G., Veiga, P., Wardwell-Scott, L. H., Tickle, T., Segata, N., Michaud, M., Gallini, C. A., Beal, C., Van Hylckama-Vlieg, J. E. T., Ballal, S. A., Morgan, X. C., Glickman, J. N., Gevers, D., Huttenhower, C. & Garrett, W. S. 2014. Gut microbiome composition and function

in experimental colitis during active disease and treatment-induced remission. *ISME J.,* 8**,** 1403-1417.

Rotz, C. A. 2004. Management to reduce nitrogen losses in animal production. *J. Anim. Sci.,* 82**,** 119-137.

Ruis, M. A., Te Brake, J. H., Engel, B., Ekkel, E. D., Buist, W. G., Blokhuis, H. J. & Koolhaas, J. M. 1997. The circadian rhythm of salivary cortisol in growing pigs: effects of age, gender, and stress. *Physiol. Behav.,* 62**,** 623-630.

Saintilan, R., Merour, I., Brossard, L., Tribout, T., Dourmad, J.-Y., Sellier, P., Bidanel, J., Van Milgen, J. & Gilbert, H. 2013. Genetics of residual feed intake in growing pigs: relationships with production traits, and nitrogen and phosphorus excretion traits. *J. Anim. Sci.,* 91**,** 2542-2554.

Sakamoto, M., Takagaki, A., Matsumoto, K., Kato, Y., Goto, K. & Benno, Y. 2009. *Butyricimonas synergistica* gen. nov., sp. nov. and *Butyricimonas virosa* sp. nov., butyric acid-producing bacteria in the family '*Porphyromonadaceae*'isolated from rat faeces. *Int. J. Syst. Evol. Microbiol.,* 59**,** 1748-1753.

Salonen, A., Lahti, L., Salojärvi, J., Holtrop, G., Korpela, K., Duncan, S. H., Date, P., Farquharson, F., Johnstone, A. M. & Lobley, G. E. 2014. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. *ISME J.,* 8**,** 2218-2230.

Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., Hutchison, C. A., Slocombe, P. M. & Smith, M. 1977. Nucleotide sequence of bacteriophage ϕX174 DNA. *Nature,* 265**,** 687-695.

Sas 2011. SAS 9.3. Statistical Analysis System.

Sattler, V., Bayer, K., Schatzmayr, G., Haslberger, A. & Klose, V. 2015. Impact of a probiotic, inulin, or their combination on the piglets' microbiota at different intestinal locations. *Benef. Microbes,* 6**,** 473-483.

Savietto, D., Berry, D. P. & Friggens, N. C. 2014. Towards an improved estimation of the biological components of residual feed intake in growing cattle. *J. Anim. Sci.,* 92**,** 467-476.

Schierack, P., Walk, N., Reiter, K., Weyrauch, K. D. & Wieler, L. H. 2007. Composition of intestinal *Enterobacteriaceae* populations of healthy domestic pigs. *Microbiology,* 153**,** 3830-3837.

Schmidt, B., Mulder, I. E., Musk, C. C., Aminov, R. I., Lewis, M., Stokes, C. R., Bailey, M., Prosser, J. I., Gill, B. P., Pluske, J. R. & Kelly, D. 2011. Establishment of normal gut microbiota is compromised under excessive hygiene conditions. *PLoS ONE,* 6**,** 1-10.

Schneeberger, M., Everard, A., Gómez-Valadés, A. G., Matamoros, S., Ramírez, S., Delzenne, N. M., Gomis, R., Claret, M. & Cani, P. D. 2015. *Akkermansia muciniphila* inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Sci. Rep.,* 5**,** 1-14.

Schwandner, R., Dziarski, R., Wesche, H., Rothe, M. & Kirschning, C. J. 1999. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J. Biol. Chem.,* 274**,** 17406-17409.

Shanks, O. C., Kelty, C. A., Archibeque, S., Jenkins, M., Newton, R. J., Mclellan, S. L., Huse, S. M. & Sogin, M. L. 2011. Community structures of fecal bacteria in cattle from different animal feeding operations. *Appl. Environ. Microbiol.,* 77**,** 2992-3001.

Shirkey, T., Siggers, R., Goldade, B., Marshall, J., Drew, M., Laarveld, B. & Van Kessel, A. 2006. Effects of commensal bacteria on intestinal morphology and expression of proinflammatory cytokines in the gnotobiotic pig. *Exp. Biol. Med.,* 231**,** 1333-1345.

Siegerstetter, S. C., Petri, R. M., Magowan, E., Zebeli, Q., Lawlor, P. G. & Metzler-Zebeli, B. U. 2016. Feed efficiency related gut microbiota profiles vary in chickens raised at two locations. *ECOFCE one day symposium.* EAAP Belfast.

Stanley, D., Hughes, R. J., Geier, M. S. & Moore, R. J. 2016. Bacteria within the gastrointestinal tract microbiota correlated with improved growth and feed conversion: Challenges

presented for the identification of performance enhancing probiotic bacteria. *Front. Microbiol.,* 7**,** 1-13.

Sterk, A., Schelegel, P., Mul, A.J., Ubbink-Blanksma, M., and Bruininx E.M.A.M. 2008. Effects of sweeteners on individual feed intake characteristics and performance in group housed weanling pigs. *J. Anim. Sci.,* 86**,** 2990-2997.

Stewart, E. J. 2012. Growing Unculturable Bacteria. *J. Bacteriol.,* 194**,** 4151-4160.

Sulabo, R. C., Tokach, M.D., Derouchey, J.M., Dritz, S.S., Goodband, R.D., and Nelssen, J.L. 2010. Influence of feed flavours and nursery diet complexity on preweaning and nursery pig performance. *J. Anim. Sci.,* 88**,** 3918- 3926.

Sun, Y., Zhou, L., Fang, L., Su, Y. & Zhu, W. 2015. Responses in colonic microbial community and gene expression of pigs to a long-term high resistant starch diet. *Front. Microbiol.,* 6**,** 877.

Suo, C., Yin, Y., Wang, X., Lou, X., Song, D., Wang, X. & Gu, Q. 2012. Effects of *Lactobacillus plantarum*ZJ316 on pig growth and pork quality. *BMC Vet. Res.,* 8**,** 1-12.

Szymanski, C. M., Yao, R., Ewing, C. P., Trust, T. J. & Guerry, P. 1999. Evidence for a system of general protein glycosylation in *Campylobacter jejuni*. *Mol. Microbiol.,* 32**,** 1022-1030.

Tako, E., Glahn, R., Welch, R., Lei, X., Yasuda, K. & Miller, D. 2008. Dietary inulin affects the expression of intestinal enterocyte iron transporters, receptors and storage protein and alters the microbiota in the pig intestine. *Br. J. Nutr.,* 99**,** 472-480.

Tan, Z., Yang, T., Wang, Y., Xing, K., Zhang, F., Zhao, X., Ao, H., Chen, S., Liu, J. & Wang, C. 2017. Metagenomic analysis of cecal microbiome identified microbiota and functional capacities associated with feed efficiency in Landrace finishing pigs. *Front. Microbiol.,* 8**,** 1-11.

Teagasc 2014. *Biosecurity procedures for visitors to pig units in Ireland*.

Teagasc. Pig farmers conference 2015. Pig Farmers Conference 2015, 2015.

Teagasc 2016. Pig herd performance report 2016. *Teagasc Pig Development Department*.

Torok, V. A., Hughes, R. J., Mikkelsen, L. L., Perez-Maldonado, R., Balding, K., Macalpine, R., Percy, N. J. & Ophel-Keller, K. 2011. Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. *Appl. Environ. Microbiol.,* 77**,** 5868-5878.

Torres-Pitarch, A., Hermans, D., Manzanilla, E. G., Bindelle, J., Everaert, N., Beckers, Y., Torrallardona, D., Bruggeman, G., Gardiner, G. E. & Lawlor, P. G. 2017. Effect of feed enzymes on digestibility and growth in weaned pigs: A systematic review and meta-analysis. *Anim. Feed Sci. Technol.,* 24**,** 1-15.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. A., Affourtit, J. P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R. & Gordon, J. I. 2009. A core gut microbiome in obese and lean twins. *Nature,* 457**,** 480-484.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R. & Gordon, J. I. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature,* 444**,** 1027-1131.

Tuśnio, A., Taciak, M., Barszcz, M., Święch, E., Bachanek, I. & Skomiał, J. 2017. Effect of Replacing Soybean Meal by Raw or Extruded Pea Seeds on Growth Performance and Selected Physiological Parameters of the Ileum and Distal Colon of Pigs. *PLoS ONE,* 12**,** 1-15.

Umu, Ö. C. O., Frank, J. A., Fangel, J. U., Oostindjer, M., Da Silva, C. S., Bolhuis, E. J., Bosch, G., Willats, W. G. T., Pope, P. B. & Diep, D. B. 2015. Resistant starch diet induces change in the swine microbiome and a predominance of beneficial bacterial populations. *Microbiome,* 3**,** 1-15.

Untea, A., Criste, R., Taranu, I., Souffrant, W., Janczyk, P., Vladescu, L. & Dragomir, C. 2013. Availability of microelements in recently weaned piglets fed diet supplemented with inulin. *Czech J. Anim. Sci.,* 58**,** 369-374.

Ursell, L. K., Clemente, J. C., Rideout, J. R., Gevers, D., Caporaso, J. G. & Knight, R. 2012. The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites. *J. Allergy Clin. Immunol.,* 129**,** 1204-1208.

Van Der Aar, P. J., Molist, F. & Van Der Klis, J. D. 2016. The central role of intestinal health on the effect of feed additives on feed intake in swine and poultry. *Anim. Feed Sci. Technol.,* 7**,** 1-12.

Varley, P. F., Mccarney, C., Callan, J. J. & O'doherty, J. V. 2010. Effect of dietary mineral level and inulin inclusion on phosphorus, calcium and nitrogen utilisation, intestinal microflora and bone development. *J. Sci. Food. Agric.,* 90**,** 2447-2454.

Veizaj-Delia, E., Piu, T., Lekaj, P., and Tafaj, M. 2010. Using combined probiotic to improve growth performance of weaned piglets on extensive farm conditions. *Livest. Sci.,* 134**,** 249-251.

Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, P., Ballew, R. M., Huson, D. H., Wortman, J. R., Zhang, Q., Kodira, C. D., Zheng, X. H., Chen, L., Skupski, M., Subramanian, G., Thomas, P. D., Zhang, J., Gabor Miklos, G. L., Nelson, C., Broder, S., Clark, A. G., Nadeau, J., Mckusick, V. A., Zinder, N., Levine, A. J., Roberts, R. J., Simon, M., Slayman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, I., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hannenhalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Abu-Threideh, J., Beasley, E., Biddick, K., Bonazzi, V., Brandon, R., Cargill, M., Chandramouliswaran, I., Charlab, R., Chaturvedi, K., Deng, Z., Francesco, V. D., Dunn, P., Eilbeck, K., Evangelista, C., Gabrielian, A. E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T. J., Higgins, M. E., Ji, R.-R., Ke, Z., Ketchum, K. A., Lai, Z., Lei, Y., Li, Z., Li, J., Liang, Y., Lin, X., Lu, F., Merkulov, G. V., Milshina, N., Moore, H. M., Naik, A. K., Narayan, V. A., Neelam, B., Nusskern, D., Rusch, D. B., Salzberg, S., Shao, W., Shue, B., Sun, J., Wang, Z. Y., Wang, A., Wang, X., Wang, J., Wei, M.-H., Wides, R., Xiao, C., Yan, C., et al. 2001. The Sequence of the Human Genome. *Science,* 291**,** 1304-1351.

Vermeire, S., Joossens, M., Verbeke, K., Wang, J., Machiels, K., Sabino, J., Ferrante, M., Van Assche, G., Rutgeerts, P. & Raes, J. 2016. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J. Crohns. Colitis.,* 10**,** 387-394.

Vigors, S., O'doherty, J. V., Kelly, A. K., O'shea, C. J. & Sweeney, T. 2016a. The effect of divergence in feed efficiency on the intestinal microbiota and the intestinal immune response in both unchallenged and Lipopolysaccharide challenged ileal and colonic explants. *PLoS ONE,* 11**,** 1-16.

Vigors, S., Sweeney, T., O'shea, C. J., Kelly, A. K. & O'doherty, J. V. 2016b. Pigs that are divergent in feed efficiency, differ in intestinal enzyme and nutrient transporter gene expression, nutrient digestibility and microbial activity. *Animal***,** 1-8.

Waite, D. W., Deines, P. & Taylor, M. W. 2013. Quantifying the impact of storage procedures for faecal bacteriotherapy in the critically endangered new zealand parrot, the kakapo (*Strigops habroptilus*). *Zoo Biol.,* 32**,** 620-625.

Walsh, A. M., Sweeney, T., Bahar, B., Flynn, B. & O'doherty, J. V. 2013. The effects of supplementing varying molecular weights of chitooligosaccharide on performance, selected microbial populations and nutrient digestibility in the weaned pig. *Animal,* 7**,** 571-579.

Walsh, M. C., Buzoianu, S. G., Gardiner, G. E., Rea, M. C., Gelencsér, E., Jánosi, A., Epstein, M. M., Ross, R. P. & Lawlor, P. G. 2011. Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. *PLoS ONE,* 6**,** 1-12.

Walsh, M. C., Buzoianu, S. G., Gardiner, G. E., Rea, M. C., Ross, R. P., Cassidy, J. P. & Lawlor, P. G. 2012. Effects of short-term feeding of Bt MON810 maize on growth performance, organ morphology and function in pigs. *Br. J. Nutr.,* 107**,** 364-371.

Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.,* 73**,** 5261-5267.

Wellock, I. J., Houdijk, J.G.M., Miller, A.C., Gill, B.P., Kyriazakis, I. 2009. The effect of weaner diet protein content and diet quality on the long term performance of pigs to slaughter. *J. Anim. Sci.,* 87**,** 1261-1269.

Wijtten, P. J., Van Der Meulen, J. & Verstegen, M. W. 2011. Intestinal barrier function and absorption in pigs after weaning: a review. *Br. J. Nutr.,* 105**,** 967-981.

Wilkinson, J. M. 2011. Re-defining the efficiency of feed use by livestock. *Animal,* 5**,** 1014-1022.

Williams, B. A., Verstegen, M.W.A., and Tamminga, S. 2001. Fermentation in the large intestine of single stomached animals and its relationship to animal health. *Nutr. Res. Rev.,* 14**,** 207-227.

Willing, B. P., And Van Kessel, A.G. 2010. Host pathways for recognition: establishing gastrointestinal microbiota as relevant in animal health and nutrition. *Livest. Sci.,* 133**,** 82-91.

Wlodarska, M., Willing, B. P., Bravo, D. M. & Finlay, B. B. 2015. Phytonutrient diet supplementation promotes beneficial *Clostridia* species and intestinal mucus secretion resulting in protection against enteric infection. *Sci. Rep.,* 5**,** 1-9.

Wondra, K. J., Hancock, J.D., Behnk, K.C., Hines, R.H., and Stark, C.R. 1995. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. *J. Anim. Sci.,* 73**,** 757-763.

Wright, R. S., Anderson, J. W. & Bridges, S. R. 1990. Propionate inhibits hepatocyte lipid synthesis. *Proc. Soc. Exp. Biol. Med.,* 195**,** 26-29.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D. & Lewis, J. D. 2011. Linking long-term dietary patterns with gut microbial enterotypes. *Science,* 334**,** 105-108.

Xiao, L., Estellé, J., Kiilerich, P., Ramayo-Caldas, Y., Xia, Z., Feng, Q., Liang, S., Pedersen, A. Ø., Kjeldsen, N. J., Liu, C., Maguin, E., Doré, J., Pons, N., Le Chatelier, E., Prifti, E., Li, J., Jia, H., Liu, X., Xu, X., Ehrlich, S. D., Madsen, L., Kristiansen, K., Rogel-Gaillard, C. & Wang, J. 2016. A reference gene catalogue of the pig gut microbiome. *Nat. Microbiol.,* 1**,** 1-6.

Xiao, Y., Yan, H., Diao, H., Yu, B., He, J., Yu, J., Zheng, P., Mao, X., Luo, Y. & Chen, D. 2017. Early gut microbiota intervention suppresses dss-induced inflammatory responses by deactivating TLR/NLR signalling in pigs. *Sci. Rep.,* 7**,** 1-12.

Xu, M.-Q., Cao, H.-L., Wang, W.-Q., Wang, S., Cao, X.-C., Yan, F. & Wang, B.-M. 2015. Fecal microbiota transplantation broadening its application beyond intestinal disorders. *World. J. Gastroenterol.,* 21**,** 102-111.

Yadav, P. N., Liu, Z. & Rafi, M. M. 2003. A diarylheptanoid from Lesser Galangal (*Alpinia officinarum*) inhibits proinflammatory mediators via inhibition of mitogen-activated protein kinase, p44/42, and transcription factor nuclear factor-κB. *J. Pharmacol. Exp. Ther.,* 305**,** 925-931.

Yan, H., Diao, H., Xiao, Y., Li, W., Yu, B., He, J., Yu, J., Zheng, P., Mao, X., Luo, Y., Zeng, B., Wei, H. & Chen, D. 2016. Gut microbiota can transfer fiber characteristics and lipid metabolic profiles of skeletal muscle from pigs to germ-free mice. *Sci. Rep.,* 6**,** 1-12.

Yang, F., Hou, C., Zeng, X. & Qiao, S. 2015. The use of lactic acid bacteria as a probiotic in Swine diets. *Pathogens,* 4**,** 34-45.

Yang, H., Huang, X., Fang, S., He, M., Zhao, Y., Wu, Z., Yang, M., Zhang, Z., Chen, C. & Huang, L. 2017. Unraveling the fecal microbiota and metagenomic functional capacity associated with feed efficiency in pigs. *Front. Microbiol.,* 8**,** 1-11.

Yang, H., Huang, X., Fang, S., Xin, W., Huang, L. & Chen, C. 2016. Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness. *Sci. Rep.,* 6**,** 1-11.

Yang, Y. X., Jin, Z., Yoon, S.Y., Choi, J.Y., Shinde, P.L., Piao, X.S., Kim, B.W., Ohh, S.J., and Chae, B.J. 2008. Lysine restriction during grower phase on growth performance, blood metabolites, carcass traits and pork quality in grower finisher pigs *Acta Agric. Scand. A Anim. Sci.,* 58**,** 14-22.

Yasuda, K. 2007. *Effects of inulin on iron utilization by young anemic pigs and implications for human nutrition.* Master of Science, Cornell University.

Yasuda, K., Maiorano, R., Welch, R. M., Miller, D. D. & Lei, X. G. 2007. Cecum is the major degradation site of ingested inulin in young pigs. *J. Nutr.,* 137**,** 2399-2404.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., Knight, R. & Gordon, J. I. 2012. Human gut microbiome viewed across age and geography. *Nature,* 486**,** 222-227.

Young, R. J. & Lawrence, A. B. 1994. Feeding behaviour of pigs in groups monitored by a computerised feeding system. *Anim. Prod.,* 58**,** 145-152.

Zarling, E. J. & Ruchim, M. A. 1987. Protein origin of the volatile fatty acids isobutyrate and isovalerate in human stool. *J. Lab. Clin. Med.,* 109**,** 566-570.

Zeng, B., Han, S., Wang, P., Wen, B., Jian, W., Guo, W., Yu, Z., Du, D., Fu, X., Kong, F., Yang, M., Si, X., Zhao, J. & Li, Y. 2015. The bacterial communities associated with fecal types and body weight of rex rabbits. *Sci. Rep.,* 5**,** 1-8.

Zhang, Q., Widmer, G., and Tzipori, S. 2013. A pig model of the human gastrointestinal tract. *Gut Microbes,* 43**,** 193-200.

Zhao, W., Wang, Y., Liu, S., Huang, J., Zhai, Z., He, C., Ding, J., Wang, J., Wang, H., Fan, W., Zhao, J. & Meng, H. 2015. The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. *PLoS ONE,* 10**,** 1-13.

Zhou, Z., Zheng, W., Shang, W., Du, H., Li, G. & Yao, W. 2015. How host gender affects the bacterial community in pig feces and its correlation to skatole production. *Ann. Microbiol.,* 65**,** 2379-2386.

Zhu, C., Fuchs, C. D., Halilbasic, E. & Trauner, M. 2016. Bile acids in regulation of inflammation and immunity: friend or foe? *Clin. Exp. Rheumatol.,* 34**,** 25-31.

Zimmermann, J. A., Fusari, M. L., Rossler, E., Blajman, J. E., Romero-Scharpen, A., Astesana, D. M., Olivero, C. R., Berisvil, A. P., Signorini, M. L., Zbrun, M. V., Frizzo, L. S. & Soto, L. P. 2016. Effects of probiotics in swines growth performance: A meta-analysis of randomized controlled trials. *Anim. Feed Sci. Technol.,* 219**,** 280-293.

Acknowledgments

I wish to express my sincere gratitude to my supervisors, Dr. Peadar Lawlor and Dr. Gillian Gardiner, for allowing me to do this PhD and for their constant guidance and for passing on their knowledge and skills to me. Thank you to Peadar for always having time for a chat, and to Gillian, for all the late nights spent correcting my work. I would like to acknowledge the European Union's Seventh Framework Programme and Teagasc Walsh fellowship for funding the work done in this thesis.

To my fellow pig students; Jen, Oceane, Susan, Hazel and Fiona, thank you for the laughs, tears, and the never ending supply of chocolate and jokes. Special thanks to Alberto, Maria, and Alessia, for teaching me all about your countries (and reminding me how bad the weather is in Ireland), and to my pig office family (Phoebe and John), for the constant support, encouragement and laughter over the past few months. Thank you to Kathryn for 2 years of craziness, and for supplying comfort in the form of food and laughter. To Kaitlynn, thank you for your never-ending encouragement, despite the many miles and oceans between us. I have been very lucky to have made friends for life during this PhD.

To everyone in the pig department; especially Tomas, Pat, John and Henry, thank you for teaching me much more than I would have thought possible. And to John Heffernan, thank you for finding me Harley. Thank you to Donagh Berry, Fiona Crispie, Orla O'Sullivan and Paul Cotter, everyone in WIT, especially Mari and Orla O'Donovan. To Stefan and Tania, thank you for your guidance and help over the past few years. To the ECOFCE project, especially Barbara, Henry and Elizabeth, thank you for all the kind help you have given to me over the course of the project. I would like to thank all of the work placement students that have helped me over the years, most especially Gwynneth and Stuart who were an incredible help. Special thanks to Chris Creevey and Toby

Wilkinson in Aberystwyth University, for your help with bioinformatics and your willingness to answer any and all of my emails over the past few months.

To my friends, now scattered across Ireland and the world, thank you for the support and love, and for always having something kind to say, and understanding when I didn't have the time to meet over the past 3 and a half years.

Finally, to my wonderful family, who never quite understood my love for the pigs or what exactly I was doing (faecal sampling is not something to discuss over dinner!). To my dog Harley, who deserves this PhD as much as I do, for spending all the late nights in the office and sacrificing walks so I could finish my thesis on time. Although she cannot read this, without her I would not have survived the past few months of stress. To Aidan, David, Rachel, and most especially Leah, thank you for your support and love, always. To Noel, who sadly passed away during this PhD, thank you for always asking how my pigs were, long after they went to the factory. To my Dad, for always encouraging my love for animals and my amazing step-mother Edel, you are my hero; thank you for all that you do.

Appendix

Publications, conferences attendance and presentations

Journal publications

- McCormack UM, Curião T, Buzoianu SG, Prieto ML, Ryan T, Varley P, Crispie F, Magowan E, Metzler-Zebeli BU, Berry DP, O'Sullivan O, Cotter PD, Gardiner GE, and Lawlor PG (2017). **Exploring a possible link between the intestinal microbiota and feed efficiency in pigs.** *Applied and Environmental Microbiology* doi:10/1128/AEM.00380-17.

- McCormack UM, Curião T, Metzler-Zebeli BU, Magowan E, Berry DP, Reyer H, Prieto ML, Harrison M, Rebeiz N, Buzoianu SG, Crispie F , Cotter PD, O'Sullivan O, Gardiner GE, and Lawlor PG (2017). **Porcine feed efficiency (FE)-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake (RFI).** *Submitted to Microbiome December 2017.*

- McCormack UM, Curião T, Wilkinson T, Metzler-Zebeli BU, Reyer H, Calderon-Diaz JA, Ryan T, Crispie F, Cotter PD, Creevey CJ, Gardiner GE, and Lawlor PG (2017). **Shifts in the intestinal microbiome and other physiological parameters in response to faecal microbiota transplantation in sows and their offspring.** *Submitted to Scientific Reports June 2017.*

- McCormack UM, Curião T, Wilkinson T, Metzler-Zebeli BU, Reyer H, Crispie F, Cotter PD, Creevey CJ, Gardiner GE, and Lawlor PG (2017). **Improving feed efficiency through microbial modulation: faecal microbiota transplantation in sows and inulin feeding in piglets.** *Intended for submission to Applied and Environmental Microbiology July 2017*

- Metzler-Zebeli BU, Lawlor PG, McCormack UM, Curiao T, Hollmann M, Ertl R, Aschenbach JR, Zebeli Q. (2017). **Finishing pigs that are divergent in feed efficiency show small differences in intestinal functionality and structure.** *PloS one* 10.1371/journal.pone.0174917.

Conference abstracts and short papers (oral presentations)

- McCormack UM, Curião T, Metzler-Zebeli BU, Magowan E, Berry DP, Reyer H, Prieto ML, Harrison M, Rebeiz N, Buzoianu SG, Crispie F , Cotter PD, O'Sullivan O, Gardiner GE, and Lawlor PG (2017). **Porcine feed efficiency (FE)-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake (RFI).** In proceedings of the British Society of Animal Science (BSAS), Chester, England.

- McCormack UM, Curião T, Metzler-Zebeli BU, Magowan E, Berry DP, Reyer H, Prieto ML, Harrison M, Rebeiz N, Buzoianu SG, Crispie F , Cotter PD, O'Sullivan O, Gardiner GE, and Lawlor PG (2017). **Porcine feed efficiency (FE)-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake (RFI).** In proceedings of the Importance of nutrition and environment on birth weight, muscle growth, health and survival of the neonate conference, Teagasc Moorepark, Ireland

- McCormack UM, Curião T, Magowan E, Metzler-Zebeli BU, Buzoianu SG, Berry DP, Cotter PD, O' Sullivan O, Crispie F, Reyer H, Gardiner GE and Lawlor PG (2016). **Growth and intestinal microbiota differ in pigs ranked on RFI on three European sites.** Presented at the 67th Annual Meeting of the European Federation of Animal Science (EAAP), Belfast, Northern Ireland

- McCormack UM, Curião T, Metzler-Zebeli BU, Buzoianu SG, Cotter PD, O' Sullivan O, Crispie F, Gardiner GE and P.G. Lawlor (2016). **Faecal microbiota transplant alters the growth and intestinal bacterial profile of pigs.**

Presented at the 67th Annual Meeting of the European Federation of Animal Science (EAAP), Belfast, Northern Ireland

- Curião T, McCormack UM, Metzler-Zebeli BU, Buzoianu SG, Berry DP, Cotter PD, O' Sullivan O, Crispie F, Gardiner GE and P.G. Lawlor (2016). **Composition of the intestinal microbiota varies between high and low RFI pigs.** Presented at the 67th Annual Meeting of the European Federation of Animal Science (EAAP), Belfast, Northern Ireland

- McCormack UM, Buzoianu SG, Berry DP, Varley P, Cotter P, O'Sullivan O, Gardiner GE and P.G Lawlor (2015). **Investigating the potential of the intestinal microbiota to impact feed efficiency in pigs.** In proceedings of Waterford Institute of technology (WIT) research day 2015, Co. Waterford, Ireland (page 22)

- Buzoianu SG, McCormack UM, Berry DP, Gardiner GE, Magowan E, Mansoor F, Metzler-Zebeli BU, Varley P, and Lawlor PG (2015). **Growth performance from day 42 post-weaning to slaughter at ~100 kg body weight in pigs divergent for residual feed intake reared at different sites across Europe.** In proceedings of the British Society of Animal Science (BSAS), Chester, England. (page 235)

- McCormack UM, Buzoianu SG, Berry DP, Gardiner GE, Magowan E, Mansoor F, Metzler-Zebeli BU, and Lawlor PG. (2015). **Effect of ranking pigs on residual feed intake (RFI) on growth and other measurements for feed**

efficiency. In proceedings of the Agricultural Research Forum, Tullamore. Co. Offaly, Ireland. (page 87)

- Buzoianu SG, McCormack UM, Gardiner GE, Berry DP, Varley P, Cotter PD, O'Sullivan O and Lawlor PG. (2015). **High-throughput sequencing of the faecal microbiome in pigs divergent for residual feed intake.** In proceedings of the Agricultural Research Forum, Tullamore. Co. Offaly, Ireland. (page 78)

Conference abstracts and short papers (Posters)

- McCormack UM, Curiao T, Magowan E, Metzler-Zebeli BU, Buzoianu SG, Berry DP, Cotter PD, O' Sullivan O, Crispie F, Reyer H, Gardiner GE and. Lawlor PG (2016). **Growth and intestinal microbiota differ in pigs ranked on RFI on three European sites.** Teagasc pig dissemination day 2016, Co. Cavan and Co. Cork, Ireland

- McCormack UM, Buzoianu SG, Berry DP, Cotter PD, O'Sullivan O, Varley P, Magowan E, Metzler-Zebeli BU, Gardiner GE, Lawlor PG (2015). **Investigating the potential of the intestinal microbiota to impact feed efficiency in pigs.** In proceedings of the Teagasc Pig Farmers' conference, Co. Cavan and Co. Cork, Ireland and in proceedings of the Agri-Food and Biosciences Institute (AFBI) pig conference, Hillsborough, Northern Ireland (page 65)

- Buzoianu SG, McCormack UM, Gardiner GE, Berry DP, Varley P, Cotter PD, O'Sullivan O and P.G Lawlor (2015). **Profile of the faecal microbiome in pigs divergent for residual feed intake.** In poster proceedings of the Digestive physiology of pigs (DPP), Wroclaw, Poland. (poster 40)

- McCormack UM, Buzoianu SG, Berry DP, Varley P, PD Cotter, O'Sullivan O, Gardiner GE, and Lawlor PG (2015). **Investigating the potential of the intestinal microbiota to impact feed efficiency in pigs.** In proceedings of Teagasc pig research dissemination day 2015, Co. Cavan and Co. Cork, Ireland (page 39-40)