Porcine feed efficiency is associated with intestinal microbiota composition

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Intestinal microbiota and feed efficiency (FE)

- Ecosystem of **trillions of microbes** in the intestine
- **Play an essential role in the host** (health, metabolism, growth)
- **Energy harvest** from diet, so it is likely to **impact FE in pigs**
- In pig production, feed accounts for ≈ **70% of cost**
- Thus **manipulation of intestinal microbiota could enhance profitability** (*FMT-study, abst. no. 24595*)

Characterise intestinal microbiota of pigs of high, medium and low residual feed intake (RFI), with genetic, nutritional and management variations minimized
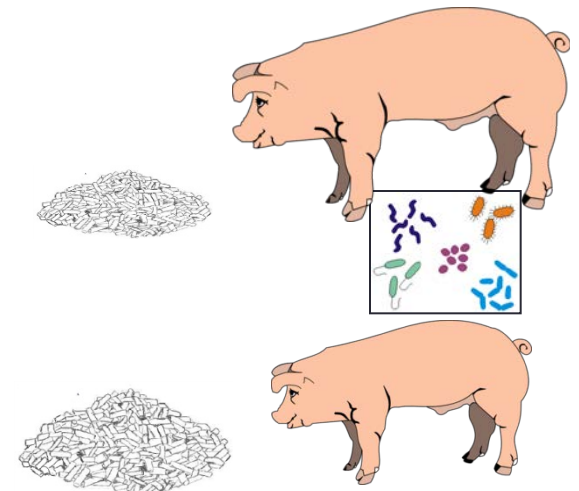
Selection of pigs and sampling

7 sows (Large White x Landrace) inseminated
with semen from 5 boars (Maxgro)
Litters kept intact to weaning

**Individually housed:
weaning to slaughter**
Same diet and environment for all pigs

RFI ranking: weaning to day 126 post weaning
32 pigs: **high** (**10**), **medium** (**10**), **low** (**12**)
Per litter, at least 1 pig per RFI rank selected

✓ **Extremes of RFI**
✓ **Low RFI = better FE**

**Microbiota composition and function &
volatile fatty acid concentrations**
Faeces: weaning, day 42 pw, day 138 pw

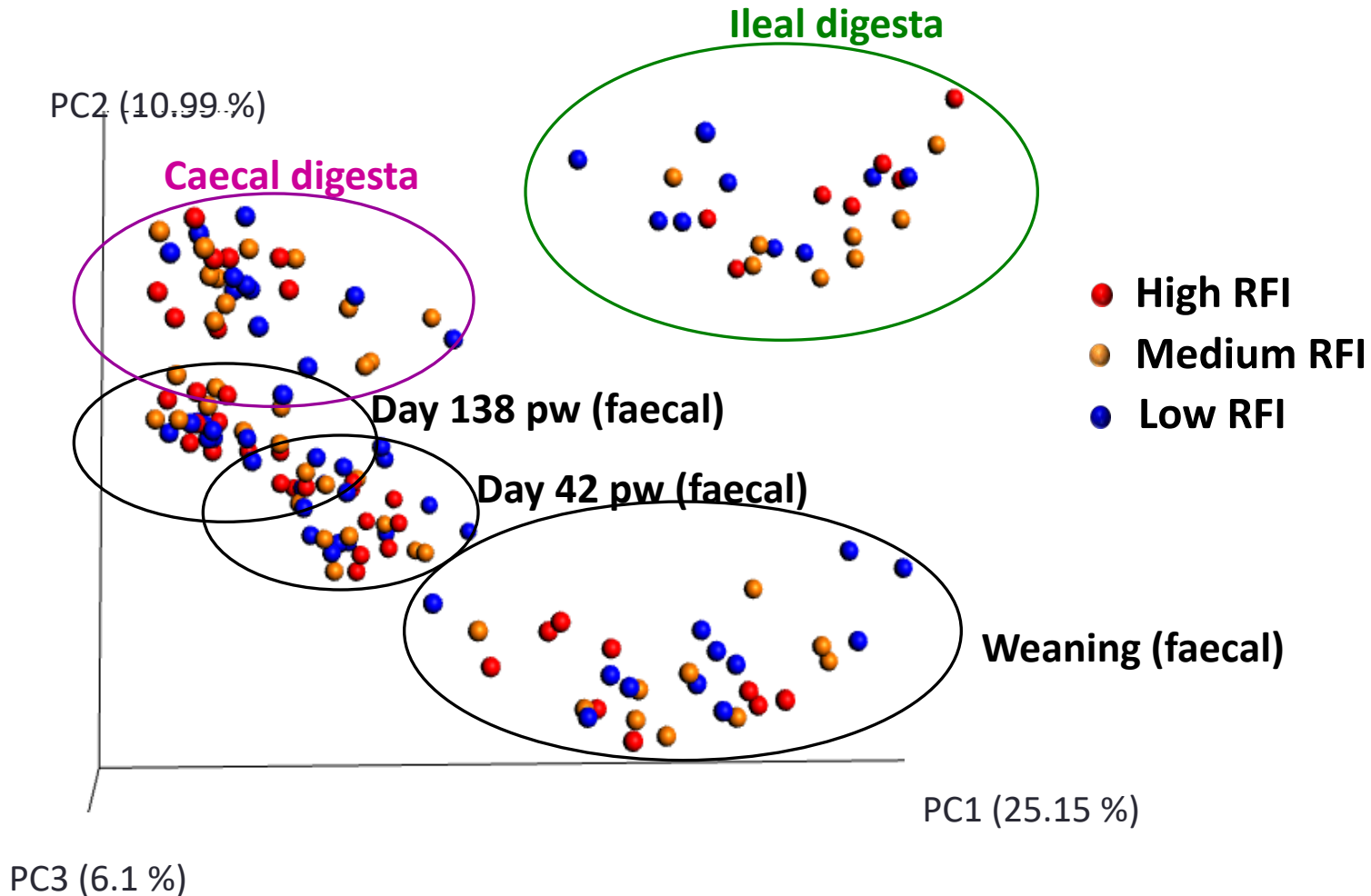Ileal and caecal digesta at slaughter

Ileal histology
Goblet cell no., villus height
& width, crypt depth

Abst. no. 24596
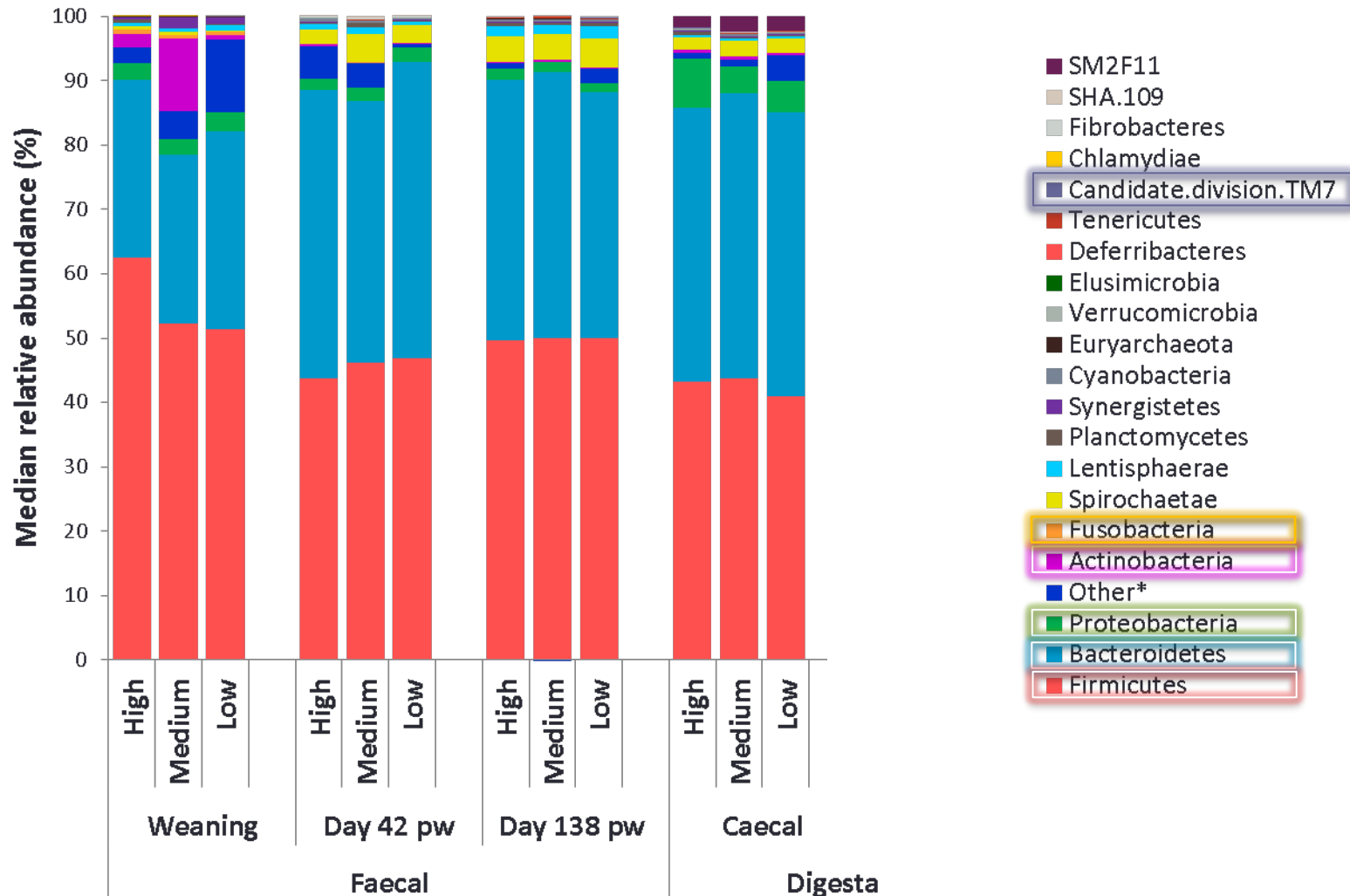
RESULTS

β-diversity varied over time and sample type

Principle coordinate analysis (PCoA) plot based on OTUs by RFI rank & sample type

α-diversity: Chao1, Simpson, Shannon indices by RFI rank, *P*>0.05

Compositional differences: phylum

*Other = No blast hits/uncultured

Differences in rel. abundance for two phyla ($P<0.05$, high and low RFI)

Compositional differences: family & genus

| Bacterial taxa (F_Family, G_Genus)			Median relative abundance (%) high vs. low RFI	Low RFI	Sample
Actinobacteria	*Actinomycetales*	F_Nocardiaceae	37 vs. 12	↓	Ileal
		G_Rhodococcus	37 vs. 12	↓	Ileal
Firmicutes	*Clostridiales*	F_Clostridiaceae1	8 vs. 4		F d138
		G_Clostridium sensu.stricto1	8 vs. 4		F d138
		G_uncultured Clostridiales			F d138
		F_Christensenellac~			F d138
		G_uncu~			F d138
				↑	F d138
			0.002 vs. 0.01	↑	Ileal
			0.06 vs. 0.03	↓	F weaning
		~cterium	0.003 vs. 0	↓	Caecal
		F_Streptococcaceae	0.01 vs. 0	↓	F d42
		G_Streptococcus	0.007 vs. 0	↓	F d42
Bacteroidetes	*Bacteroidales*	F_Bacteroidaceae	0.04 vs. 0.18	↑	F d138
		G_Bacteroides	0.04 vs. 0.18	↑	F d138
Proteobacteria	*Pasteurellales*	G_Actinobacillus	0.002 vs. 0.009	↑	Caecal
Archaea	*Methanobacteriales*	G_Methanosphaera	0.001 vs. 0.000	↓	Ileal

Biomarkers for FE?

F: faeces

Kruskal-Wallis test for independent samples and the Wilcoxon-Rank test for paired samples, *P*<0.05

Metagenomic functional prediction
PICRUSt faecal & intestinal microbiota of pigs ranked on RFI
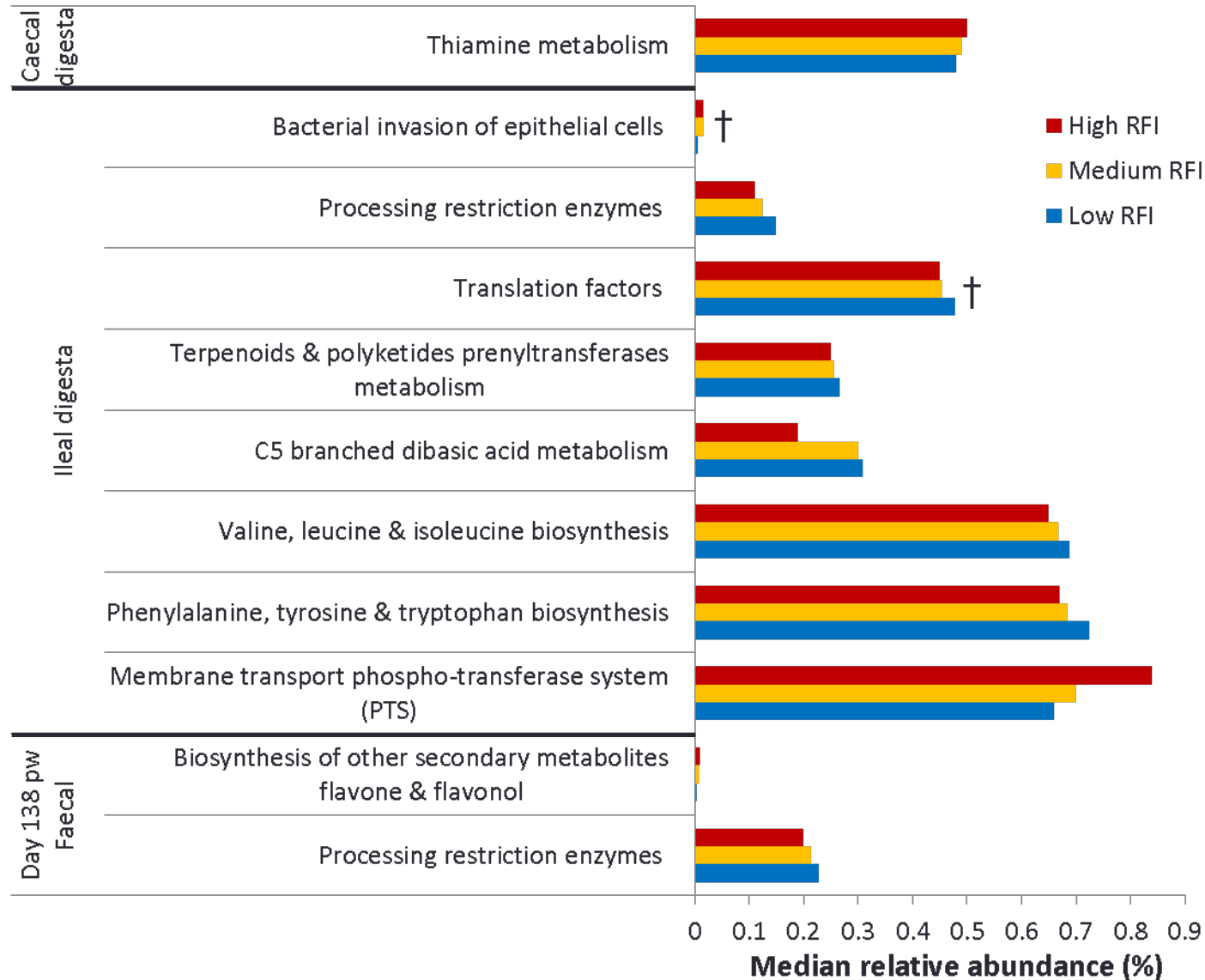
$P<0.05$
† $P<0.10$
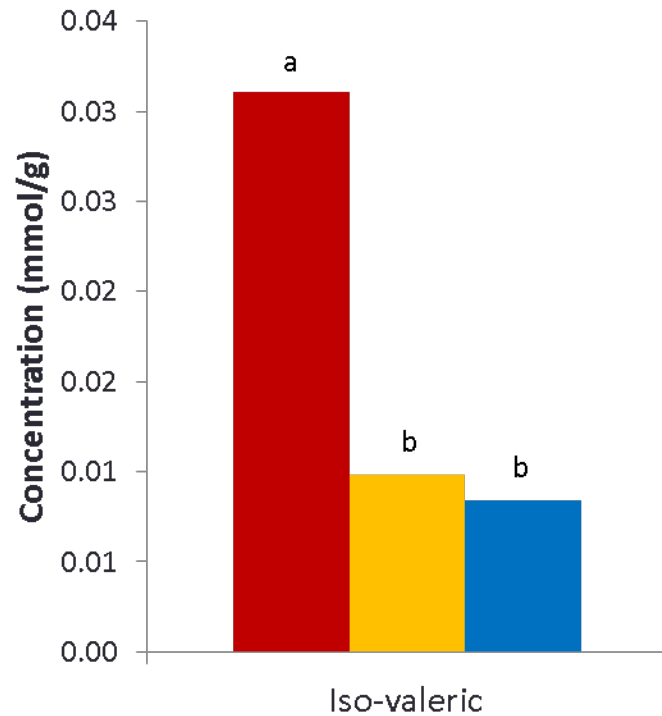
Volatile fatty acid concentrations

Faeces (weaning)   **Ileal digesta**

Legend: High RFI, Medium RFI, Low RFI
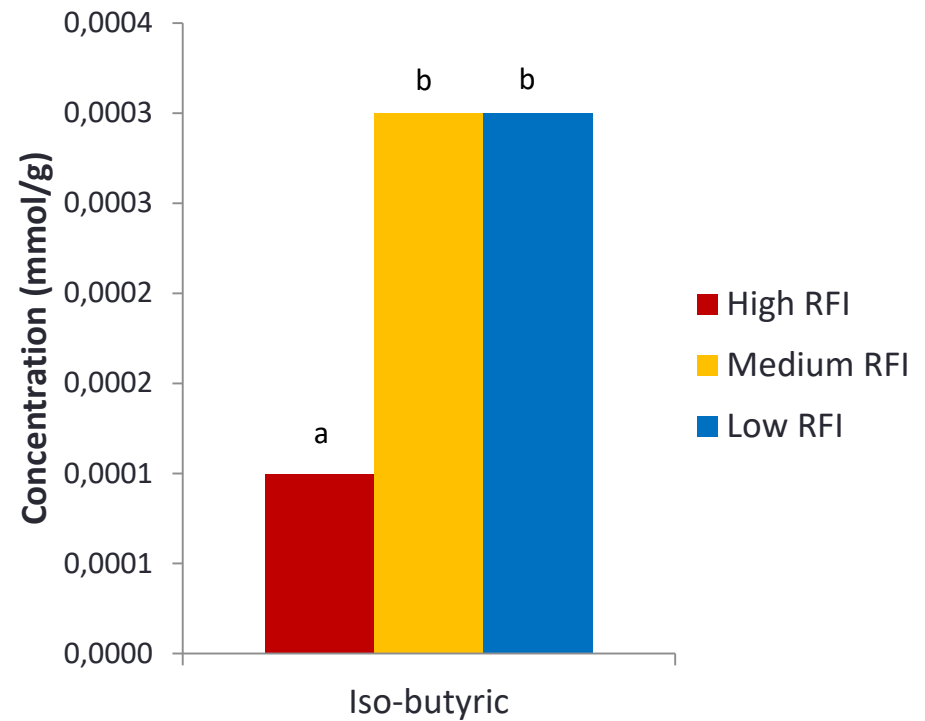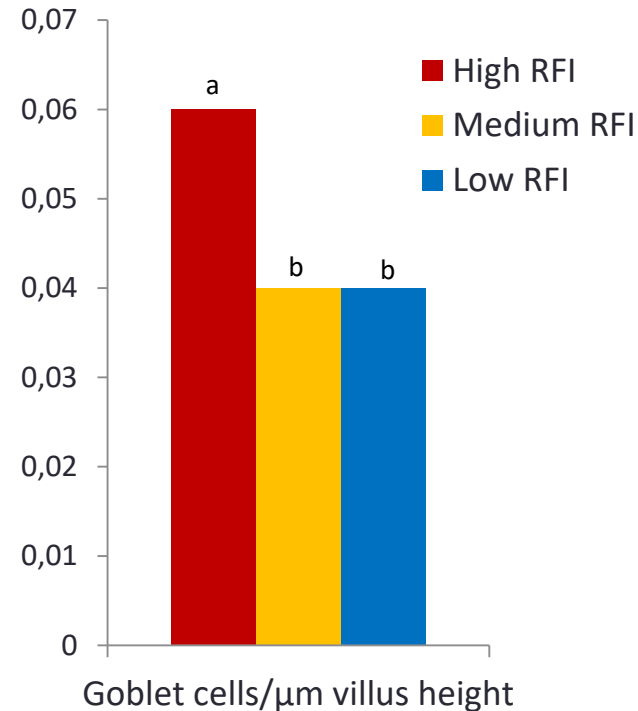
P<0.05

Ileal histology
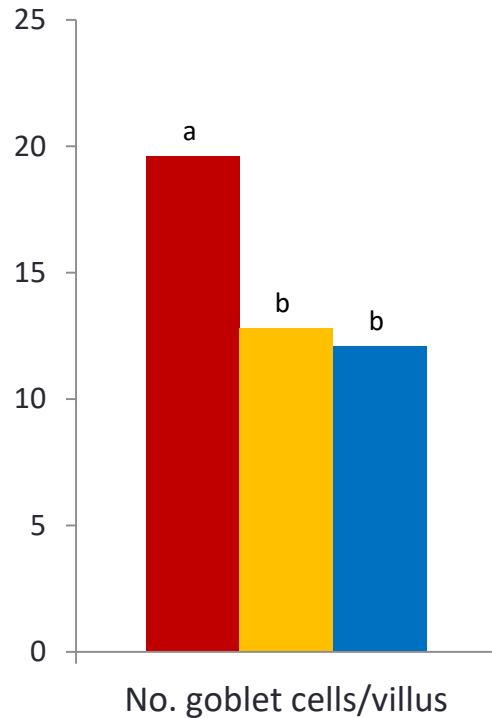
P<0.05

" Villus height:crypt depth: *P*>0.05
Goblet cells in low RFI pigs:

- " Mucin production: host defense (Liu *et al.* 2006)
- " Intestinal inflammation (Kim *et al.* 2010)
- ' nutrient absorption?

Conclusions

- **Intestinal microbiota**
  - No clustering by RFI, but samples clustered by age & intestinal site
  - RFI-associated differences: **potential biomarkers** for improved FE
    - Low RFI pigs:
      - ↑ *Christensenellaceae* family (lean phenotype)
      - ↑ *Oscillibacter* (valerate producer, health benefits)
      - ↑ *Cellulosilyticum* (cellulose degrading ability)

  - **Predicted microbial functionality (ileum):**
    - ↑ bacterial pathways related to **metabolism** (amino acids & core)
    - ↓ bacterial sugar-uptake transporters (higher CHO availability for pig utilization)

Conclusions

- **Volatile fatty acids**
  - ↓ Iso-valeric (faeces) and ↑ iso-butyric (ileum) acids (end products of protein microbial fermentation):

    Improved protein utilization?

- ↓ **Ileal goblet cells** linked with improved FE
  - ↓ mucin production: ↑ permeability and nutrient absorption?
  - Improved intestinal health- no inflammation?

  First comprehensive set of potential microbial biomarkers for FE in pigs. Microbiota is a likely driver of FE in pigs.

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Thank you

EFFICIENT & ECOLOGICALLY-FRIENDLY PIG AND POULTRY PRODUCTION.

A WHOLE-SYSTEMS APPROACH TO OPTIMISING FEED EFFICIENCY AND REDUCING THE ECOLOGICAL FOOTPRINT OF MONOGASTRICS.

ECOFCE
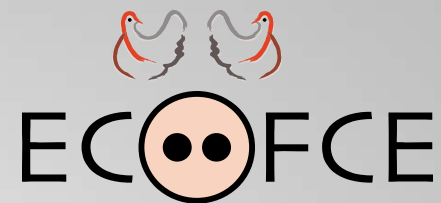
BASIC DATA

Funding:
EU-FP7
(€ 6 million)

Start date:
1 February 2013

Duration:
48 months
(2013 to 2017)

Screening process

- 81 pigs, individually housed from weaning to slaughter

  - 2 weeks before slaughter: 32 pigs from 7 litters selected as extremes in RFI

    - High RFI (10): poor feed efficiency
    - Medium RFI (10): average feed efficiency
    - Low RFI (12): good feed efficiency

Weaning
d0

d 42 pw

d 126 pw

Slaughter
d139 pw

F — P — F

P

S

F I C

Saliva (cortisol)
Ileal tissue (histology)
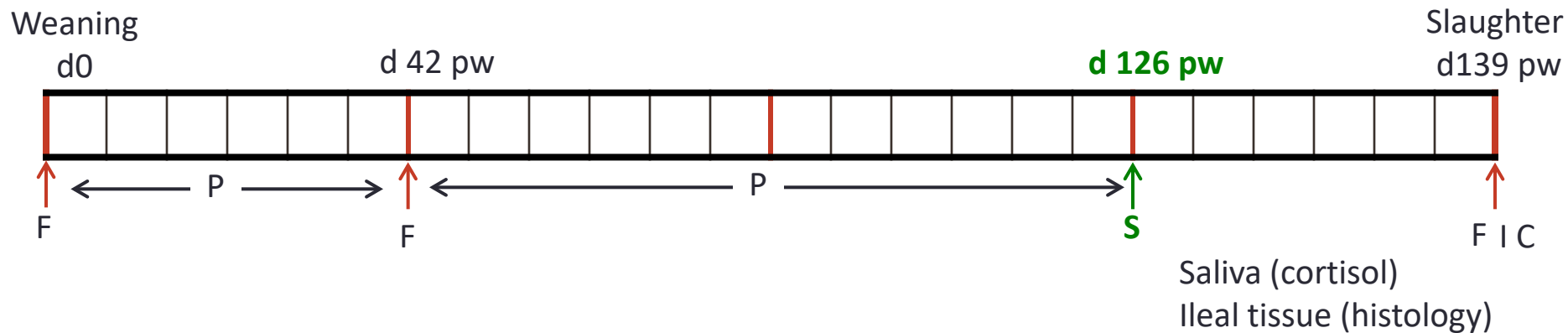
F – faecal; I – ileal digesta; C – caecal digesta; P – performance; S-selection

Growth performance (weaning to selection)

	High	Medium	Low	S.E.	P-value
RFI[1] (g)	76.0[a]	6.0[b]	-51.0[c]	15.40	<0.001
RIG[2] (g)	-1693[a]	-179[b]	986[c]	42.9	<0.001
RG[3] (g)	-12.0	-2.0	4.0	8.2	0.39
Wean weight (kg)	9.17	8.92	7.89	0.297	0.51
Selection weight (kg)	132.9	129.4	123.1	1.81	0.19
ADFI (g/day)	1850[a]	1732[a,b]	1631[b]	51.2	<0.01
ADG (g/day)	910	877	855	28.4	0.38
FCE (g/g)	1.91[a]	1.86[a,b]	1.79[b]	0.025	<0.01

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

[1]**Residual feed intake (RFI)**= the difference between observed feed intake and that predicted by growth
[2]**Residual intake and gain (RIG)**= (RG/SD RG) - (RFI/SD RFI)
[3]**Residual gain (RG)**= the difference between observed gain and that predicted by growth
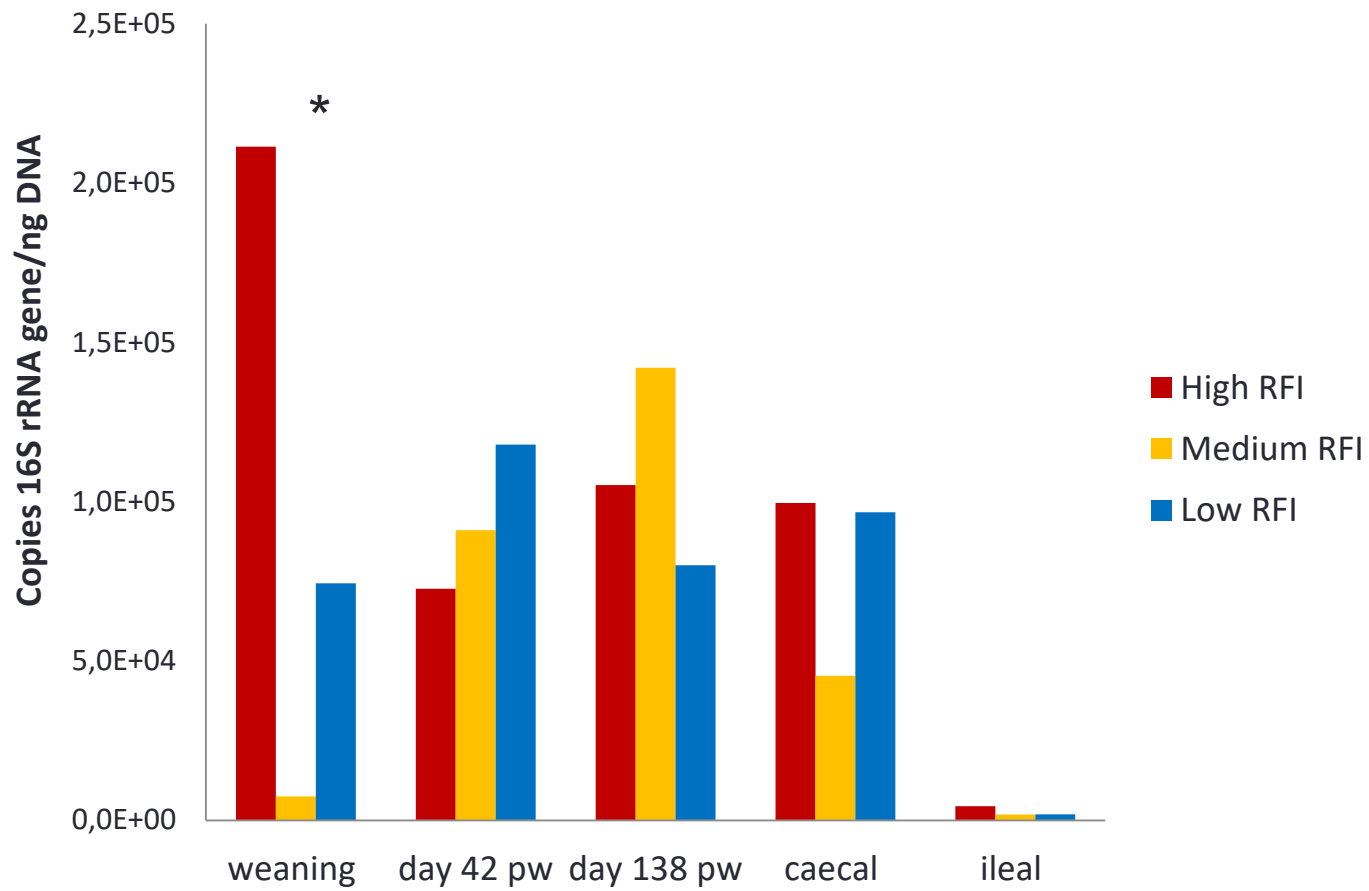
Salivary cortisol

Cortisol (pooled results from day 135 & 137 pw): stressed animals divert energy away from lean meat deposition (Richardson *et al.* 2004)

	High	Medium	Low	S.E.	P-value
Salivary cortisol (ng/ml)	4.8	4.1	4.5	1.38	0.74

Total bacterial DNA

* P<0.05

Correlations for the significantly different genus & VFA

Genus	Acetic	Propionic	Butyric	Isobutyric	Valeric	Isovaleric
Actinobacteria.Nocardiaceae.Rhodococcus				-0.164	-0.186	-0.180
Firmicutes.Clostridiaceae.Clostridium.sensu.stricto.1	-0.297	-0.291	-0.301	-0.426	-0.381	-0.364
Firmicutes.Christensenellaceae_uncultured	0.274	0.307	0.309	0.300	0.297	0.217
Firmicutes.Ruminococcaceae.Oscillibacter		-0.154	-0.164		0.139	
Firmicutes.Lachnospiraceae.Cellulosilyticum	-0.256	-0.305	-0.276	-0.356	-0.286	-0.286
Firmicutes.Streptococcaceae.Streptococcus		-0.110		-0.182	-0.155	-0.153
Bacteroidetes.Bacteroidaceae.Bacteroides	0.243	0.261	0.248	0.219	0.201	0.136
Proteobacteria.Pasteurellaceae.Actinobacillus					-0.166	-0.141

All correlations P<0.05

Phylum & VFA correlations

Phyla	Acetic	Butyric	Isobutyric	Propionic	Valeric	Isovaleric
Acidobacteria	-0.182	-0.161	0.060	-0.195	-0.143	-0.070
Actinobacteria	-0.047	-0.040	0.075	-0.019	-0.179	-0.135
Bacteroidetes	0.063	0.010	0.013	-0.007	0.213	0.173
CandidatedivisionOD1	-0.118	-0.059	0.345	-0.058	-0.080	-0.088
CandidatedivisionTM7	-0.076	-0.049	0.355	-0.096	0.049	0.017
Chlamydiae	0.081	0.137	0.009	0.088	0.169	0.218
Chlorobi	-0.086	-0.112	0.145	-0.095	-0.072	-0.105
Chloroflexi	-0.105	-0.063	0.336	-0.087	-0.029	-0.057
Cyanobacteria	-0.090	-0.116	0.355	-0.134	0.039	0.030
Deferribacteres	0.214	0.133	0.003	0.182	0.278	0.173
DeinococcusThermus	-0.110	-0.089	0.011	-0.168	-0.163	-0.157
Elusimicrobia	0.027	0.044	0.293	0.042	0.097	0.037
Fibrobacteres	-0.057	-0.067	0.354	-0.055	0.037	-0.043
Firmicutes	0.150	0.193	0.078	0.174	0.083	0.105
Fusobacteria	0.038	0.062	0.355	0.088	-0.053	-0.021
Lentisphaerae	0.095	0.171	0.006	0.122	0.172	0.100
Nitrospirae	-0.061	-0.078	0.305	-0.038	-0.002	-0.049
Planctomycetes	0.135	0.148	0.009	0.115	0.245	0.099
Proteobacteria	-0.064	-0.067	0.064	-0.078	-0.106	-0.080
SHA109	-0.025	-0.015	0.355	0.030	0.089	0.031
SM2F11	-0.094	-0.018	0.355	-0.078	-0.071	-0.057
Spirochaetae	0.014	-0.034	0.350	-0.031	0.156	0.045
Synergistetes	0.209	0.284		0.276	0.259	0.180
Tenericutes	-0.024	-0.029	0.355	-0.044	0.121	0.008
Verrucomicrobia	0.075	0.088	0.060	0.098	0.168	0.076
Eukaryota_Excavata	-0.028	-0.104	0.224	-0.036	0.013	-0.103
Archaea_Euryarchaeota		0.213				

Chapter 2: Carcass quality

	High	Medium	Low	S.E	P-value
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 (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

Chapter 2: Organ weights

	High	Medium	Low	S.E	P-value
OFFAL (g)	4022	4195	4194	105.2	0.45
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
GIT (g)	2049	2246	2257	123.3	0.44
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

Methodology

Microbial profiling

16S rRNA gene sequencing (Illumina)

OTU classification, taxonomy assignment
β- and α-diversity
Predicted functionality (PICRUSt)

Volatile fatty acid concentration

Gas chromatography

Total bacterial quantification

qPCR 16S rRNA gene

Ileal histology

Microscopic observation of ileum tissue