

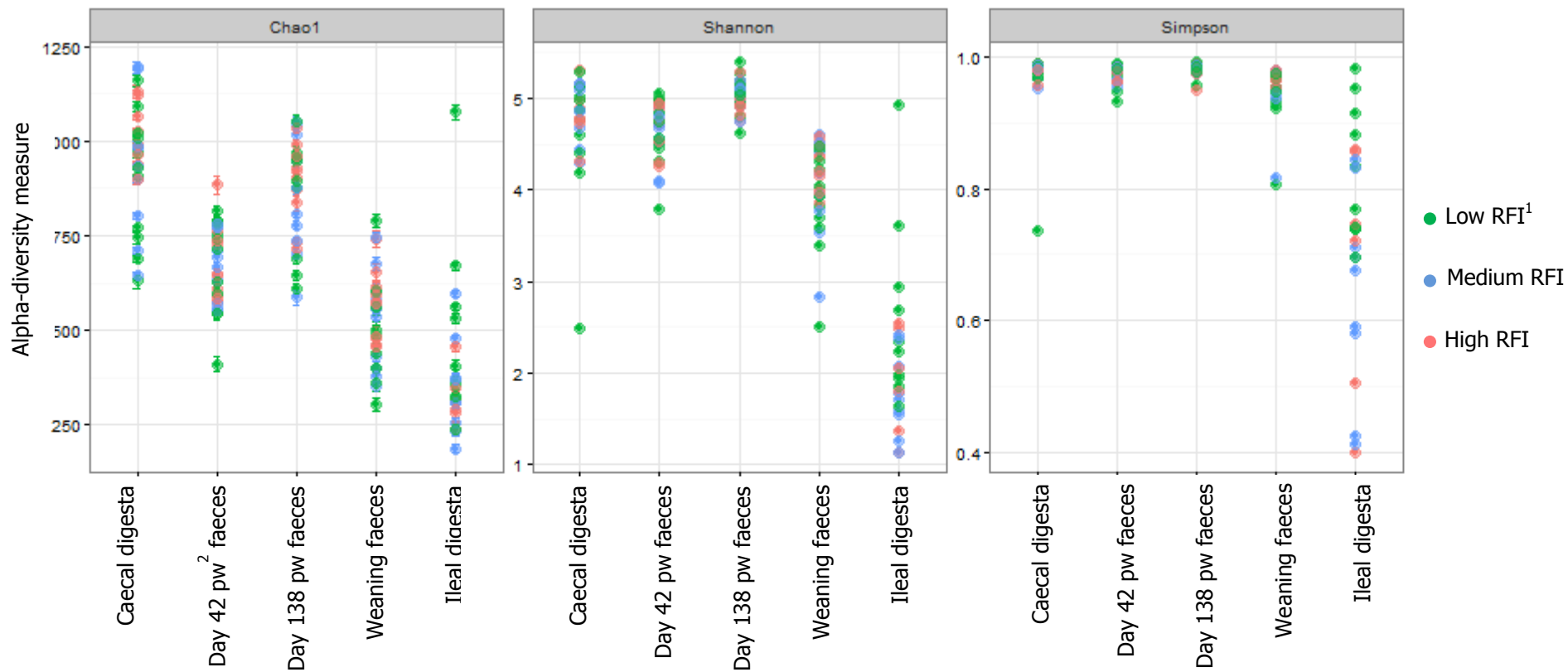
# Exploring a possible link between the intestinal microbiota and feed efficiency in pigs

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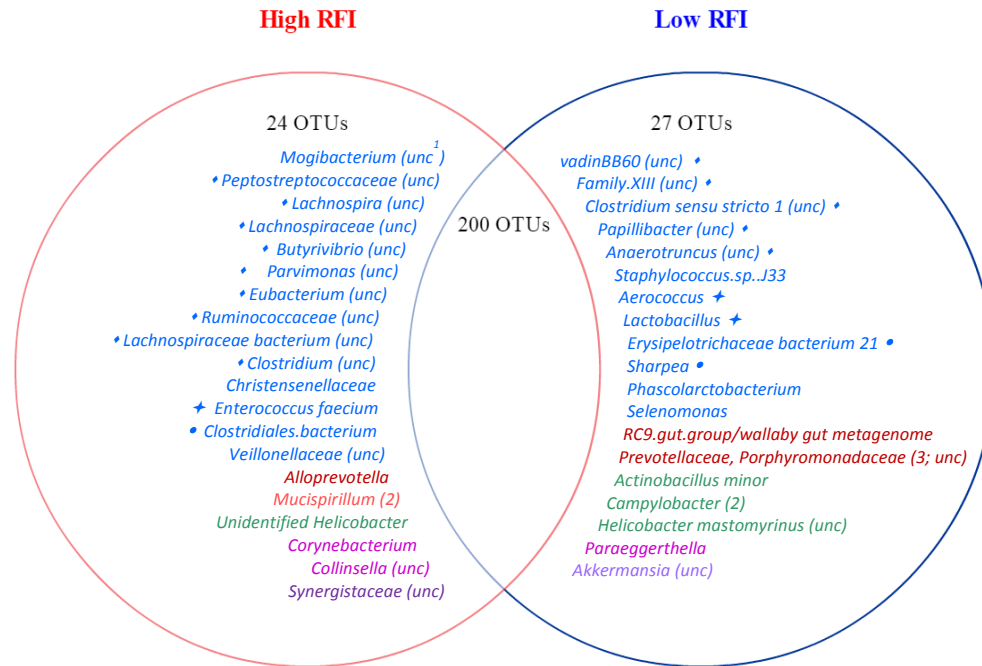
Running title: Porcine RFI-associated intestinal microbiota

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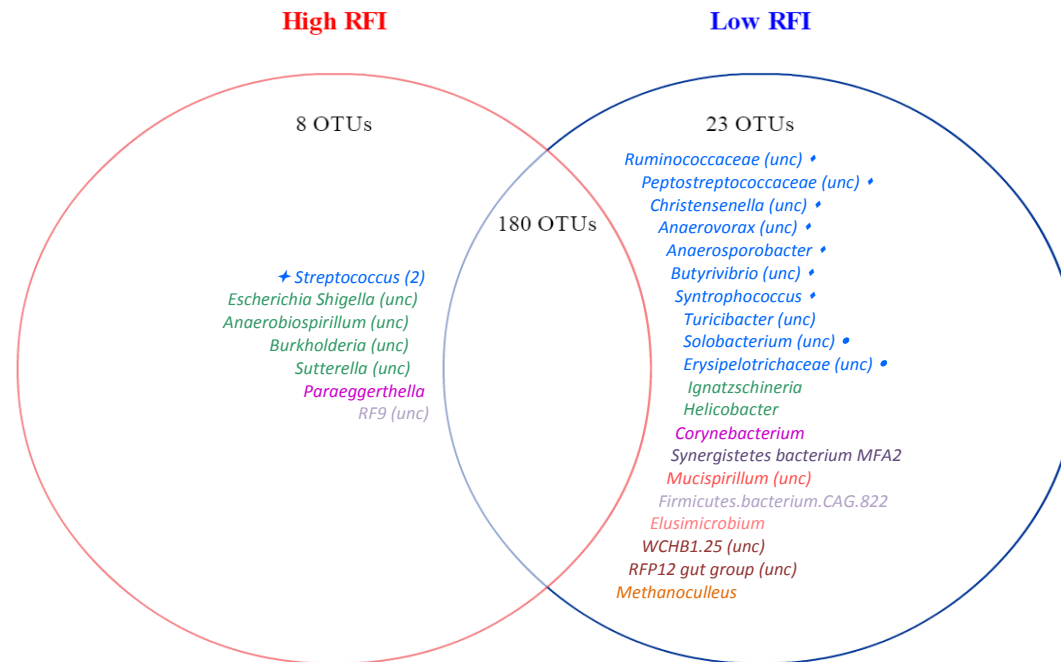


**Fig. S1.  $\alpha$ -diversity indices of the pig intestinal microbiota according to residual feed intake (RFI) rank and sample type (n=150) (colour coded by RFI rank).<sup>1</sup>RFI: residual feed intake; 2pw: post-weaning. Low RFI: faeces (n=36), digesta (caecal n=12, ileal n=9); medium RFI: faeces (n=30), digesta (caecal n=10, ileal n=9); high RFI: faeces (n=30), digesta (caecal n=8, ileal n=6).**

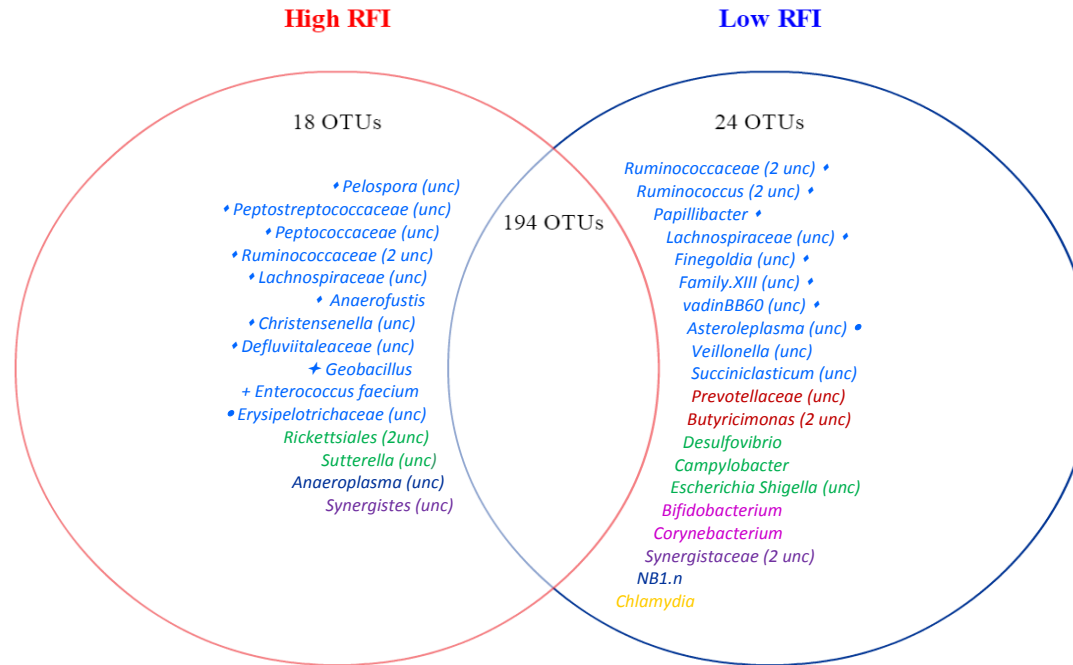
## A. Weaning faeces



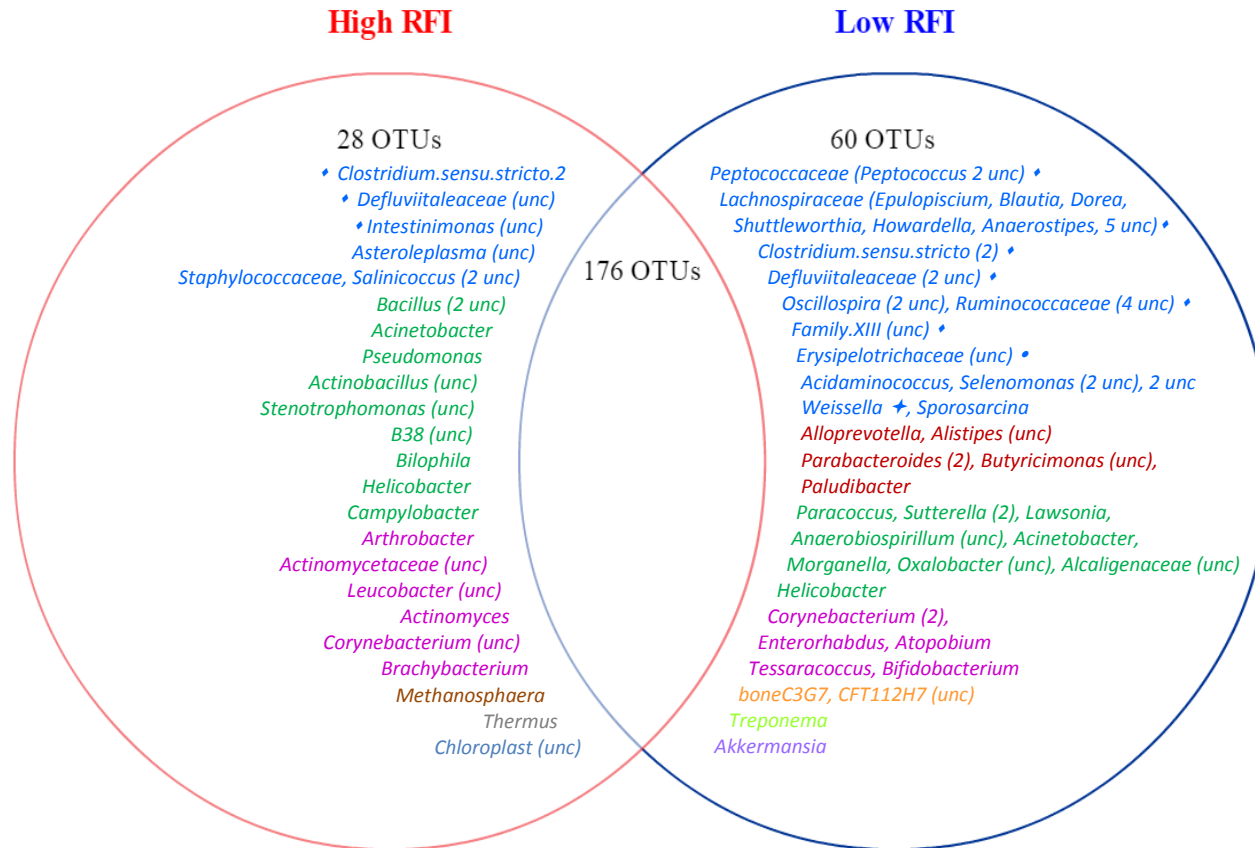
## B. Day 42 pw<sup>2</sup> faeces



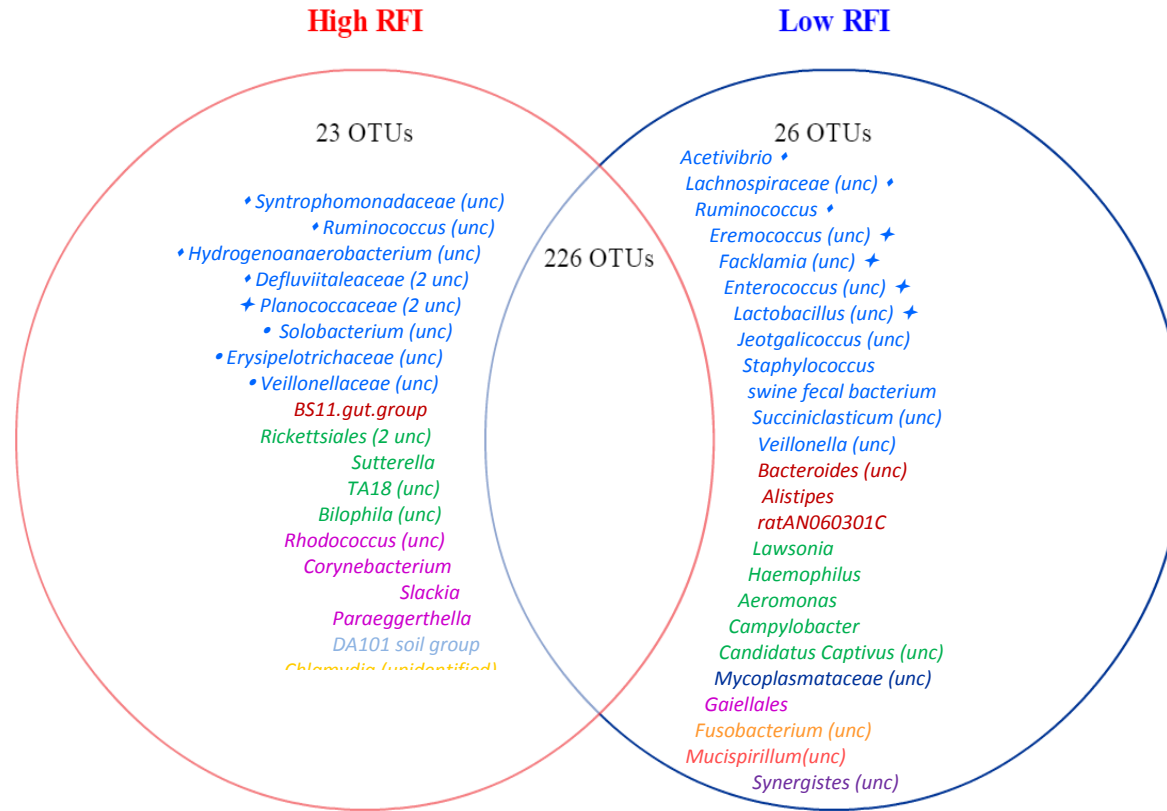
C. Day 138 pw faeces



## D. Ileal digesta



E. Caecal 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*

**Fig. S2. 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).

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



**Table S1. Composition and chemical analysis of diets used in the study (on an as-fed basis; g/kg)**

<b>Diet Type</b>	<b>Starter</b>	<b>Link</b>	<b>Weaner</b>	<b>Finisher</b>	<b>Pregnant sow</b>	<b>Lactating sow</b>
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 <sup>1</sup>	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 <sup>2</sup>	3.0 <sup>2</sup>	3.0 <sup>2</sup>	1.0 <sup>3</sup>	1.5 <sup>4</sup>	1.5 <sup>4</sup>
Natuphos 5000 FTU/g <sup>5</sup>	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
<b>Chemical analysis (g/kg dry matter)</b>						
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) <sup>6</sup>	17.9	17.0	16.9	16.0	15.9	16.3
Net energy (MJ/kg) <sup>6</sup>	11.4	10.3	10.6	9.8	9.5	10.5
<b>Amino acids (g/kg)</b>						
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

<sup>1</sup>Lactofeed 70 contains 70% lactose, 11.5% protein, 0.5% oil, 7.5% ash and 0.5% fibre (Volac, Cambridge, UK).

<sup>2</sup>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<sub>3</sub>, 1000 IU; vitamin E, 100 IU; vitamin K, 4 mg; vitamin B<sub>12</sub>, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B<sub>1</sub>, 2 mg; vitamin B<sub>6</sub>, 3 mg; Endox, 60 g.

<sup>3</sup>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<sub>3</sub>, 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin B<sub>12</sub>, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B<sub>1</sub>, 2 mg; vitamin B<sub>6</sub>, 3 mg.

<sup>4</sup> 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<sub>3</sub>, 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; vitamin B<sub>12</sub>, 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<sub>1</sub>, 2 mg; vitamin B<sub>6</sub>, 3 mg.

<sup>5</sup>Phytase; 5000 FTU/g equal to 500 FTU per kg finished feed.

<sup>6</sup>Digestible energy and net energy were calculated from book values.

**Table S2. Effect of ranking pigs on residual feed intake (between weaning and day 126 post weaning) on salivary cortisol concentrations<sup>1</sup> and organ weights**

Measure	High RFI	Medium RFI	Low RFI	S.E.M <sup>2</sup>	P
Salivary cortisol (ng/ml)	4.8	4.1	4.5	1.38	0.74
Heart (g)	476.5 <sup>A</sup>	522.2 <sup>B</sup>	482.3 <sup>A,B</sup>	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.

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

**Table S3. Effect of ranking pigs on residual feed intake (between weaning and day 126 post weaning) on ileal morphology<sup>1</sup>**

Measure	High RFI	Medium RFI	Low RFI	S.E.M <sup>2</sup>	P
Villus height ( $\mu\text{m}$ )	302.2	298.5	302.7	18.26	0.98
Villus width ( $\mu\text{m}$ )	116.8	117.6	153.3	15.89	0.18
Crypt depth ( $\mu\text{m}$ )	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 <sup>a</sup>	12.8 <sup>b</sup>	12.1 <sup>b</sup>	1.81	0.01
No. of goblet cells/ $\mu\text{m}$ villus height	0.06 <sup>a</sup>	0.04 <sup>b</sup>	0.04 <sup>b</sup>	0.006	0.02

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

<sup>1</sup>Ten villi were measured for each pig and the means were used for statistical analysis.

<sup>2</sup>Least square means and pooled standard error of means are presented.

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

**Table S4. 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**

<b>Time point</b>	<b>High RFI</b>	<b>Medium RFI</b>	<b>Low RFI</b>	<b>S.E.M<sup>1</sup></b>	<b>P</b>
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).

<sup>1</sup>Least square means and pooled standard error of means are presented.

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

Supplementary Table S5. Relative abundance of OTUs statistically different<sup>1</sup> among RFI ranks

Sample type	OTU Taxonomy	Median relative abundance [confidence interval] <sup>2</sup>			P-value <sup>3</sup>			
		High RFI (n=10)	Medium RFI (n=10)	Low RFI (n=12)	High vs Low	High vs Medium	Low vs Medium	
Faeces weaning	<i>Firmicutes;Clostridia;Clostridiales;Family.XIII; Mogibacterium;uncultured.bacterium</i>	0.0 [0.0;1e-05]	3e-05 [1e-05;8e-05]	0.0 [0.0;0.0]	0.53	0.06	0.04	
	<i>Firmicutes;Bacilli;Lactobacillales;Streptococcaceae; Streptococcus.;uncultured.bacterium</i>	1e-05 [0.0;1e-04]	0.0 [0.0;0.0]	4e-05 [2e-05;6e-05]	0.46	0.16	0.02	
	<i>Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae; dgA.11.gut.group;uncultured.bacterium</i>	0.00133 [0.00084;0.00397]	0.00018 [0.00005;0.00035]	0.00145 [0.00045;0.00448]	0.79	0.03	0.03	
	<i>Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae; Parabacteroides;uncultured.bacterium</i>	0.00896 [0.00401;0.01362]	0.01522 [0.01102;0.02123]	0.00721 [0.00305;0.01178]	0.74	0.12	0.02	
	<i>Firmicutes;Clostridia;Clostridiales;Peptococcaceae; Peptococcus;uncultured.organism</i>	0.00006 [0.0;0.00013]	0.0 [0.0;0.0]	0.00000 [0.00;0.00002]	0.17	0.05	0.18	
	<i>Candidate.division.TM7;Unknown.Class;Unknown.Order; Unknown.Family;Candidatus.Saccharimonas;uncultured.bacterium</i>	0.0 [0.0;0.00001]	0.00015 [0.00003;0.00018]	0.0 [0.0;0.00001]	0.90	0.07	0.07	
	Faeces day 42 pw <sup>4</sup>	<i>Firmicutes;Clostridia;Clostridiales;Ruminococcaceae; Intestinimonas;uncultured.bacterium</i>	0.00001 [0.0;0.00003]	0.00005 [0.00004;0.00014]	0.00002 [0.0;0.00009]	0.39	0.01	0.23
		<i>Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae; Alistipes;.bacterium</i>	0.0 [0.0;0.00003]	0.00006 [0.00002;0.00014]	0.00001 [0.0;0.00004]	0.38	0.03	0.16
		<i>Firmicutes;Negativicutes;Selenomonadales;Veillonellaceae; Selenomonas;uncultured.bacterium</i>	0.00191 [0.00107;0.00457]	0.00041 [0.00010;0.00075]	0.00083 [0.00031;0.00274]	0.21	0.06	0.21
Faeces day 138 pw		<i>Firmicutes;Clostridia;Clostridiales;Lachnospiraceae; uncultured;uncultured.Firmicutes.bacterium</i>	2e-05 [0e+00;3e-05]	0.0 [0.0;0.0]	0.0 [0e+00;1e-05]	0.12	0.01	0.12
	<i>Firmicutes;Clostridia;Clostridiales;Clostridiaceae.1; Clostridium.sensu.stricto.1;uncultured.Clostridiaceae.bacterium</i>	0.00017 [0.00011;0.00026]	0.00003 [0.00002;0.00008]	0.00007 [0.00004;0.00015]	0.20	0.02	0.21	
	<i>Candidate.division.TM7;Unknown.Class;Unknown.Order; Unknown.Family;Candidatus.Saccharimonas;uncultured.bacterium</i>	0.00045 [0.00026;0.00077]	0.00044 [0.00027;0.00060]	0.00015 [0.0;0.00028]	<b>0.03</b>	0.82	0.09	
	<i>Firmicutes;Clostridia;Clostridiales;Lachnospiraceae; Cellulosilyticum;uncultured.bacterium</i>	0.00014 [0.00008;0.00026]	0.00064 [0.00038;0.00092]	0.00060 [0.00026;0.00105]	<b>0.05</b>	0.05	0.92	
	<i>Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides; uncultured.bacterium</i>	0.00041 [0.00018;0.00108]	0.00142 [0.00104;0.00363]	0.00175 [0.00062;0.00431]	<b>0.04</b>	0.07	0.79	
	<i>Firmicutes;Clostridia;Clostridiales;Clostridiaceae.1; Clostridium.sensu.stricto.1;uncultured.bacterium</i>	0.08039 [0.05077;0.11418]	0.07249 [0.06478;0.08706]	0.03572 [0.03190;0.06637]	<b>0.04</b>	0.82	0.10	
	<i>Firmicutes;Clostridia;Clostridiales;vadinBB60;uncultured.organism;</i>	0.00229 [0.00132;0.00317]	0.00209 [0.00115;0.00271]	0.00513 [0.00302;0.00628]	<b>0.05</b>	0.82	0.08	

	<i>Firmicutes; Clostridia; Clostridiales; Christensenellaceae; uncultured; uncultured.bacterium</i>	0.02067 [0.01509;0.03598]	0.03442 [0.01922;0.06740]	0.05353 [0.03997;0.06825]	<b>0.04</b>	0.30	0.32
<b>Caecal digesta</b>	<i>Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; uncultured; uncultured.Clostridiales.bacterium</i>	0.00013 [0.00009;0.00034]	0.00011 [0.00004;0.00022]	0.0 [0.0;0.00003]	<b>0.002</b>	0.33	0.02
	<i>Firmicutes; Erysipelotrichia; Erysipelotrichales; Erysipelotrichaceae; Solobacterium; uncultured.bacterium</i>	0.00002 [0.0;0.00013]	0.0 [0.0;0.0]	0.0 [0.0;0.0]	<b>0.01</b>	0.07	0.27
	<i>Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Actinobacillus; Actinobacillus.porcinus</i>	0.0 [0.0;1e-05]	4e-05 [0.0;6e-05]	3e-05 [1e-05;8e-05]	<b>0.04</b>	6.80E-02	0.89
<b>Ileal Digesta</b>	<i>Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; uncultured; uncultured.Clostridiales.bacterium</i>	0.0 [0.0;0.0]	0.0 [0.0;0.0]	2e-05 [0.0;1e-04]	0.08	0.22	0.02
	<i>Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanobrevibacter; uncultured.methanogenic.archaeon</i>	1e-05 [0.0;2e-05]	5e-05 [3e-05;9e-05]	0.0 [0.0;1e-05]	0.15	0.15	0.02
	<i>Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillibacter; uncultured.bacterium</i>	0.00002 [0.00001;0.00004]	0.00009 [0.00006;0.00011]	0.00010 [0.00005;0.00022]	<b>0.03</b>	0.01	0.93
	<i>Actinobacteria; Actinobacteria; Corynebacteriales; Nocardiaceae; Rhodococcus; unidentified</i>	0.37417 [0.24461;0.54878]	0.42184 [0.09561;0.54106]	0.11800 [0.03774;0.29465]	<b>0.04</b>	0.91	0.04
	<i>Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Incertae.Sedis; Eubacterium.fissicatena</i>	2e-05 [1e-05;2e-05]	0.0 [0.0;0.0]	1e-05 [0e+00;2e-05]	0.32	0.05	0.07
	<i>Spirochaetae; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema; Treponema.berlinense</i>	0.0 [0.0;0.0]	0.0 [0.0;4e-05]	3e-05 [1e-05;4e-05]	<b>0.02</b>	0.30	0.30
	<i>Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanosphaera; uncultured.archaeon</i>	1e-05 [0.0;4e-05]	0.0 [0.0;0.0]	0.0 [0.0;0.0]	<b>0.05</b>	0.18	0.18
	<i>Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Anaerosporobacter; uncultured.bacterium</i>	1e-05 [0.0;4e-05]	0.0 [0.0;0.0]	0.0 [0.0;2e-05]	0.77	0.03	0.03
	<i>Firmicutes; Bacilli; Bacillales; Planococcaceae; uncultured; uncultured.bacterium</i>	0.00005 [<0.00001;0.00036]	0.0 [0.0;0.0]	0.0 [0.0;<0.00001]	0.11	0.07	0.42

<sup>1</sup>Statistical significance at P≤0.05.

<sup>2</sup>The values presented are not percentages; differentially abundant OTUs for which median relative abundance was less than 0.00001 are not presented.

<sup>3</sup>P-values in bold correspond to statistical differences between high and low RFI pigs.

<sup>4</sup>pw: post-weaning.

**Table S6. Effect of ranking pigs on residual feed intake (between weaning and day 126 post weaning) on pH and volatile fatty acid (VFA) concentrations ( $\mu\text{mol/g}$ ) in faeces at weaning, day 42 and day 138 post weaning**

Measure	Weaning					Day 42 post weaning					Day 138 post weaning				
	High RFI	Medium RFI	Low RFI	S.E.M <sup>1</sup>	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 <sup>2</sup>	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 <sup>A,B</sup>	22.0 <sup>B</sup>	33.3 <sup>A</sup>	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.

<sup>1</sup>Least square means and pooled standard error of the mean are presented.

<sup>2</sup>Total: Sum of measured acids.

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



**Table S7. Effect of ranking pigs on residual feed intake (between weaning and day 126 post weaning) on pH and volatile fatty acid (VFA) concentrations ( $\mu\text{mol/g}$ ) 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 <sup>1</sup>	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 <sup>2</sup>	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 <sup>a,b</sup>	3.4 <sup>b</sup>	7.2 <sup>a</sup>	0.96	0.02	5.5	4.7	3.9	0.81	0.34
Isobutyric	1.5 <sup>b</sup>	2.6 <sup>a,b</sup>	3.5 <sup>a</sup>	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.

<sup>1</sup>Least square means and pooled standard error of the mean are presented.

<sup>2</sup>Total: sum of measured acids.

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