

1 **Seeking to improve feed efficiency in pigs through microbial modulation via fecal**
2 **microbiota transplantation in sows and dietary supplementation of offspring with inulin**

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19 Running title: Microbial modulation to alter feed efficiency in pigs

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Table S1. Significant differences in parameters in offspring due to the effect of the interaction between fecal microbiota transplantation (FMT) in sows and dietary supplementation of offspring with inulin for 42 days post-weaning¹

Sow treatment	Control ²		FMTp ³		S.E.M.	Interaction	P-value	
	Control ⁴	Inulin ⁵	Control	Inulin			Sow	Offspring
Weaning								
<i>G_Streptococcus</i>	0.73 ^b	0.31 ^b	2.24 ^a	3.71 ^a	0.134	<0.001	<0.001	0.68
<i>G_Butyricimonas</i>	0.04 ^b	0.21 ^b	0.08 ^b	2.56 ^a	0.018	<0.001	<0.001	<0.001
<i>G_Bifidobacterium</i>	0.09 ^b	0.07 ^b	0.10 ^b	2.07 ^a	0.027	0.001	0.04	0.03
<i>G_Faecalibacterium</i>	0.89 ^a	0.20 ^b	0.23 ^b	0.16 ^b	0.099	<0.001	0.003	0.001
<i>G_Dorea</i>	0.20 ^{a,b}	0.57 ^a	0.03 ^b	0.31 ^a	0.009	<0.001	0.01	<0.001
<i>G_Terrisporobacter</i>	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								
<i>P_Proteobacteria</i>	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								
Body weight (kg)	59.0 ^a	58.6 ^a	51.2 ^b	53.7 ^{a,b}	1.31	<0.001	<0.001	0.41
<i>P_Actinobacteria</i>	0.19 ^b	0.64 ^{a,b}	1.15 ^a	0.50 ^{a,b}	0.170	0.004	0.03	0.76
<i>G_Campylobacter</i>	1.07 ^b	0.85 ^b	3.22 ^a	1.38 ^{a,b}	0.567	<0.001	0.002	0.01
<i>G_Peptococcus</i>	0.08 ^b	1.18 ^a	0.95 ^a	0.19 ^b	0.032	<0.001	0.49	0.24
<i>G_Bifidobacterium</i>	0.03 ^{a,b}	0.04 ^{a,b}	0.23 ^a	0.01 ^b	0.004	0.006	0.39	0.05
<i>G_Sutterella</i>	0.002 ^b	0.127 ^a	0.158 ^a	0.189 ^a	0.0003	0.004	0.005	0.01
Day 130								
<i>P_Fibrobacteres</i>	0.37 ^a	0.35 ^a	0.01 ^b	0.39 ^a	0.001	<0.001	<0.001	0.008

Sow treatment	Control ²		FMTp ³		S.E.M.	P-value		
	Control ⁴	Inulin ⁵	Control	Inulin		Interaction	Sow	Offspring
<i>G_Sphaerochaeta</i>	18.00 ^a	9.96 ^{a,b}	4.51 ^b	13.38 ^{a,b}	2.442	0.008	0.07	0.41
<i>G_Oribacterium</i>	2.76 ^a	1.80 ^{a,b}	0.73 ^b	1.80 ^{a,b}	0.434	0.004	0.01	0.37
<i>G_Blautia</i>	1.39 ^{a,b}	1.36 ^{a,b}	0.61 ^b	2.06 ^a	0.374	0.006	0.13	0.02
<i>G_Faecalitalea</i>	0.42 ^a	0.20 ^{a,b}	0.06 ^b	0.27 ^{a,b}	0.031	0.001	0.02	0.29
<i>G_Fibrobacter</i>	0.367 ^a	0.351 ^a	0.004 ^b	0.092 ^a	0.001	<0.001	<0.001	0.008
<i>G_Bifidobacterium</i>	0.03 ^b	0.46 ^{a,b}	0.82 ^a	0.30 ^{a,b}	0.007	0.004	0.02	0.15
<i>G_Acetanaerobacterium</i>	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
Day 140								
Body weight (kg)	104.9 ^a	103.9 ^a	94.3 ^b	96.8 ^b	1.29	<0.001	<0.001	0.59
Carcass weight (kg)	81.0 ^a	80.1 ^a	72.1 ^b	75.0 ^b	1.62	0.01	<0.001	0.54
Carcass 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
White blood cells ($\times 10^3$ cells/ μ L)	28.4 ^a	21.6 ^b	24.7 ^{a,b}	26.2 ^{a,b}	1.54	0.01	0.78	0.09
Hemoglobin (g/dL)	13.9 ^{a,b}	13.6 ^{a,b}	12.7 ^b	14.0 ^a	0.39	0.05	0.31	0.16
Day 35 – 140								
RFI ⁷ (g/day)	13.7 ^a	25.2 ^a	-13.9 ^b	-20.9 ^b	15.96	0.05	0.02	0.88

Sow treatment	Control ²		FMTp ³		S.E.M.	P-value		
	Control ⁴	Inulin ⁵	Control	Inulin		Interaction	Sow	Offspring
Jejunum								
Number of goblet cells per μm villus height	0.14 ^{a,b}	0.13 ^b	0.16 ^a	0.16 ^a	0.007	0.001	<0.001	0.93
Ileum								
<i>G_Prevotella</i>	0.36 ^b	3.35 ^a	2.99 ^a	4.54 ^a	0.164	<0.001	0.004	0.002
<i>G_Sphaerochaeta</i>	2.78 ^a	0.32 ^b	0.30 ^b	1.04 ^b	0.143	<0.001	0.16	0.19
<i>G_Chlamydia</i> 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 ^b	0.13 ^a	0.08 ^b	0.14 ^a	0.063	<0.001	0.76	<0.001
Glycosphingolipid biosynthesis - globo series	0.18 ^b	0.24 ^a	0.19 ^b	0.26 ^a	0.159	<0.001	0.36	<0.001
Nitrotoluene degradation	0.08 ^{a,b}	0.06 ^b	0.10 ^a	0.08 ^{a,b}	0.047	0.008	0.02	0.01
Biosynthesis of ansamycins	0.15 ^{a,b}	0.14 ^b	0.17 ^{a,b}	0.18 ^a	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 ^a	1.43 ^b	1.60 ^b	1.34 ^b	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 ^b	0.001 ^b	0.006 ^{a,b}	0.009 ^a	0.0005	<0.001	<0.001	0.64
Various types of N-glycan biosynthesis	0.0022 ^a	0.0009 ^{a,b}	0.0005 ^b	0.0013 ^{a,b}	0.00025	0.001	0.04	0.69
1,2,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	0.002 ^a	0.001 ^b	0.001 ^b	0.002 ^a	0.0002	0.002	0.04	0.69
Acetic acid ($\mu\text{mol/g}$ digesta)	14.9 ^b	10.9 ^c	10.4 ^c	16.7 ^a	1.65	0.02	0.85	0.52
Propionic acid	5.5 ^a	1.8 ^b	4.1 ^{ab}	3.9 ^{ab}	0.83	0.005	0.28	0.01
Cecum								
<i>P_Proteobacteria</i>	3.79 ^b	4.44 ^{a,b}	7.50 ^a	5.60 ^{a,b}	2.832	0.009	0.003	0.95
<i>G_Bacteroides</i>	1.27 ^b	1.57 ^b	6.77 ^a	1.58 ^b	0.715	<0.001	0.006	0.04
<i>G_Flavonifractor</i>	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 ^a	0.56 ^b	0.533	<0.001	0.17	0.11
D-alanine metabolism	0.29 ^a	0.28 ^a	0.27 ^b	0.29 ^a	0.251	<0.001	<0.001	0.009
Phenylalanine, tyrosine and tryptophan biosynthesis	1.29 ^{a,b}	1.36 ^{a,b}	1.41 ^a	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

Sow treatment	Control ²		FMTp ³		S.E.M.	P-value		
	Control ⁴	Inulin ⁵	Control	Inulin		Interaction	Sow	Offspring
Butyric acid (μmol/g digesta)	9.5 ^a	9.8 ^a	6.9 ^b	1.5 ^c	1.02	<0.001	<0.001	0.01
Valeric acid (μmol/g digesta)	6.1 ^b	8.9 ^a	6.6 ^b	4.7 ^c	0.92	0.01	0.04	0.89
Isovaleric acid (μmol/g digesta)	2.4 ^b	2.9 ^b	5.3 ^a	1.4 ^c	0.54	<0.001	0.83	0.003
Colon								
<i>P_Lentisphaerae</i>	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
Isobutyric acid (μmol/g digesta)	4.4 ^b	3.9 ^b	13.6 ^a	6.5 ^b	2.14	0.02	0.01	0.15

¹Least square means and pooled standard error of the mean are presented. Sows: ²Control (n=11) and ³FMT procedure (FMTp; n=11); Piglets: ⁴Control (n=16), ⁵Inulin (n=16) for the first 6 weeks post-weaning. P_ indicates phylum; G_ indicates genus, ⁶Also reflects relative abundance at phylum level (i.e. *Chlamydiae*). Compositional and predicted functional differences are depicted as median relative abundance values. ⁷RFI: residual feed intake measured between day 35 and 140 of age.

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

Table S2. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on number of operational taxonomic units in the offspring feces and digesta

Timepoint/ intestinal site	Control sow		FMTp sow		Control sow	FMTp sow	Control offspring	Inulin offspring
	Control offspring	Inulin offspring	Control offspring	Inulin offspring				
Day 28 (Weaning)	301	294	346	304	406	440	448	410
Day 50	148	180	221	148	226	266	266	232
Day 65	29	23	20	32	42	47	45	44
Day 100	387	302	337	336	438	417	446	400
Day 130	335	375	297	358	460	431	421	467
Ileum	43	27	31	36	44	45	48	38
Cecum	165	129	229	179	217	275	275	220
Colon	227	230	247	194	321	322	336	304

Table S3. Composition and chemical analysis of all diets used in the study (on an as-fed basis; 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

Diet Type	Starter		Link		Weaner		Finisher	Sow	
	Control	Inulin	Control	Inulin	Control	Inulin		Pregnant	Lactation
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

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

²Premix provided per Kg of complete diet: Copper sulphate 7H₂O, 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 D₃, 1000 IU; vitamin E, 100 IU; vitamin K, 4 mg; vitamin B₁₂, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B₁, 2 mg; vitamin B₆, 3 mg; Endox, 60 g.

³ Premix provided per Kg of complete diet: Copper sulphate 7H₂O, 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 D₃, 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin B₁₂, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B₁, 2 mg; vitamin B₆, 3 mg.

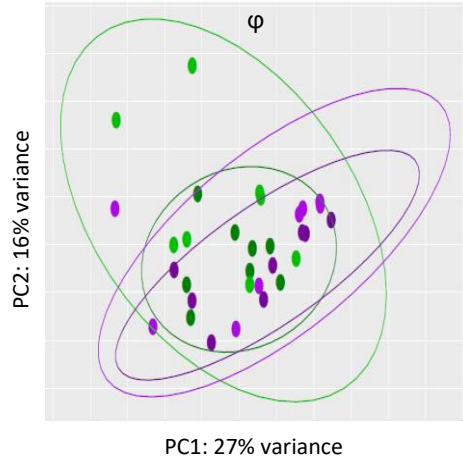
⁴ Premix provided per Kg of complete diet: Copper sulphate 7H₂O, 60 g; Ferrous sulphate monohydrate, 200 g; Manganese oxide, 80 g; Zinc oxide, 100 g; Potassium iodate, 1 g; Sodium selenite, 0.4 g; Cu, 15 mg; Fe, 70 mg; Mn, 62 mg; Zn, 80 mg, I, 0.6 mg; Se, 0.2 mg; vitamin A, 1000 IU; vitamin D₃, 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; vitamin B₁₂, 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 B₁, 2 mg; vitamin B₆, 3 mg.

⁵ Phytase: Natuphos – BASF; 5000 FTU/gm equal to 500 FTU per Kg finished feed.

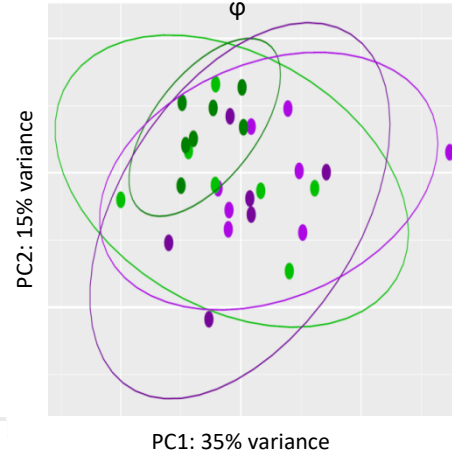
⁶Digestible energy is megajoules per kilogram of dry matter (DE= 4168-9.1*ASH +1.9*CP+3.9*EE-3.6*NDF)

⁷Calculated values

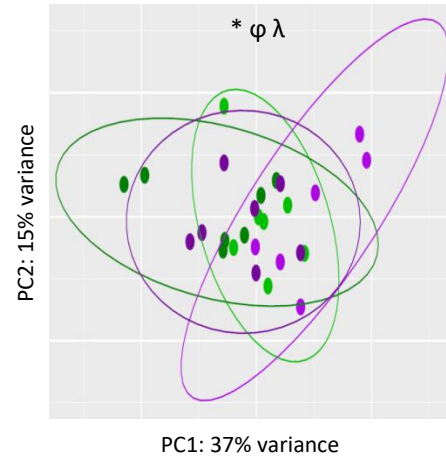
A. Feces at weaning



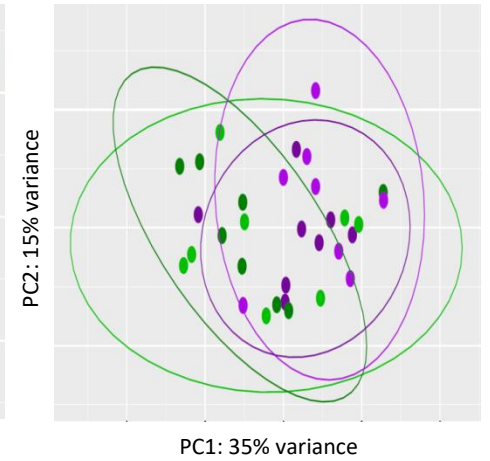
B. Feces at 50 days old



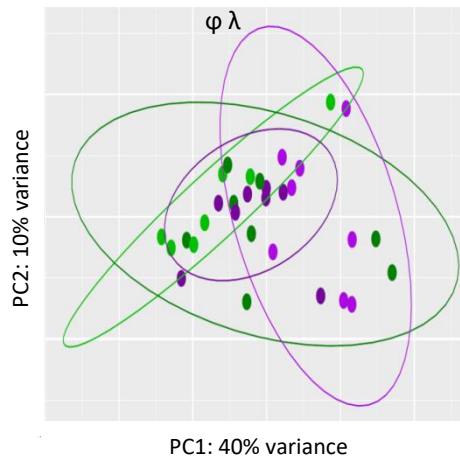
C. Feces at 65 days old



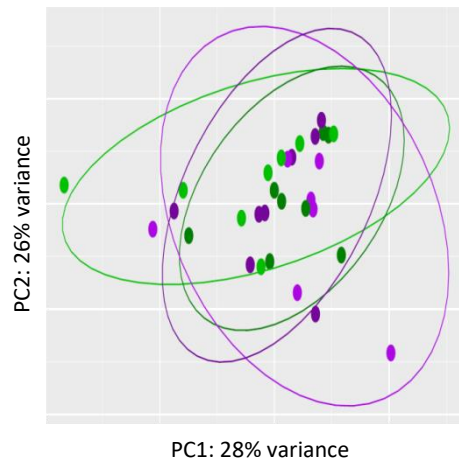
D. Feces at 100 days old



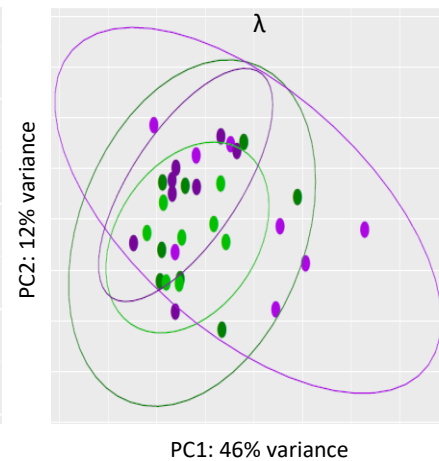
E. Feces at 130 days old



F. Ileal digesta



G. Cecal digesta



H. Colon digesta

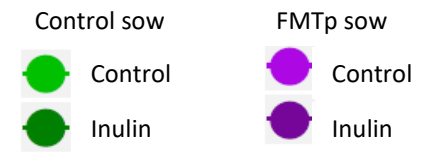
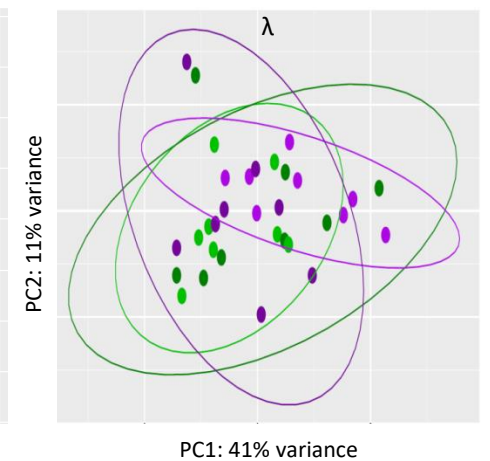
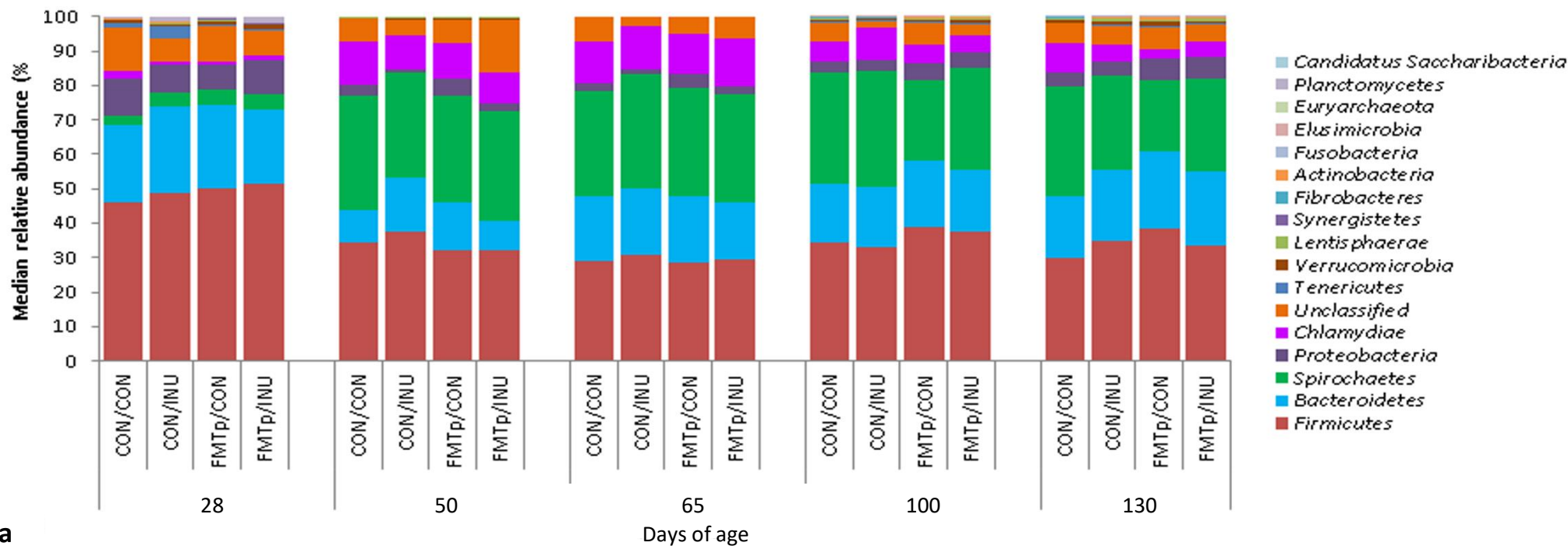


Fig S1. Microbial diversity variation due to fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning in the offspring feces at A. 28, B. 50, C. 65, D. 100 and E. 130 days of age and in the offspring F. ileum, G. cecum and H. colon, 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 x offspring treatment level ($P \leq 0.05$); ϕ indicates sow treatment effect ($P \leq 0.05$); λ indicates offspring treatment effect ($P \leq 0.05$).

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

A. Feces



B. Digesta

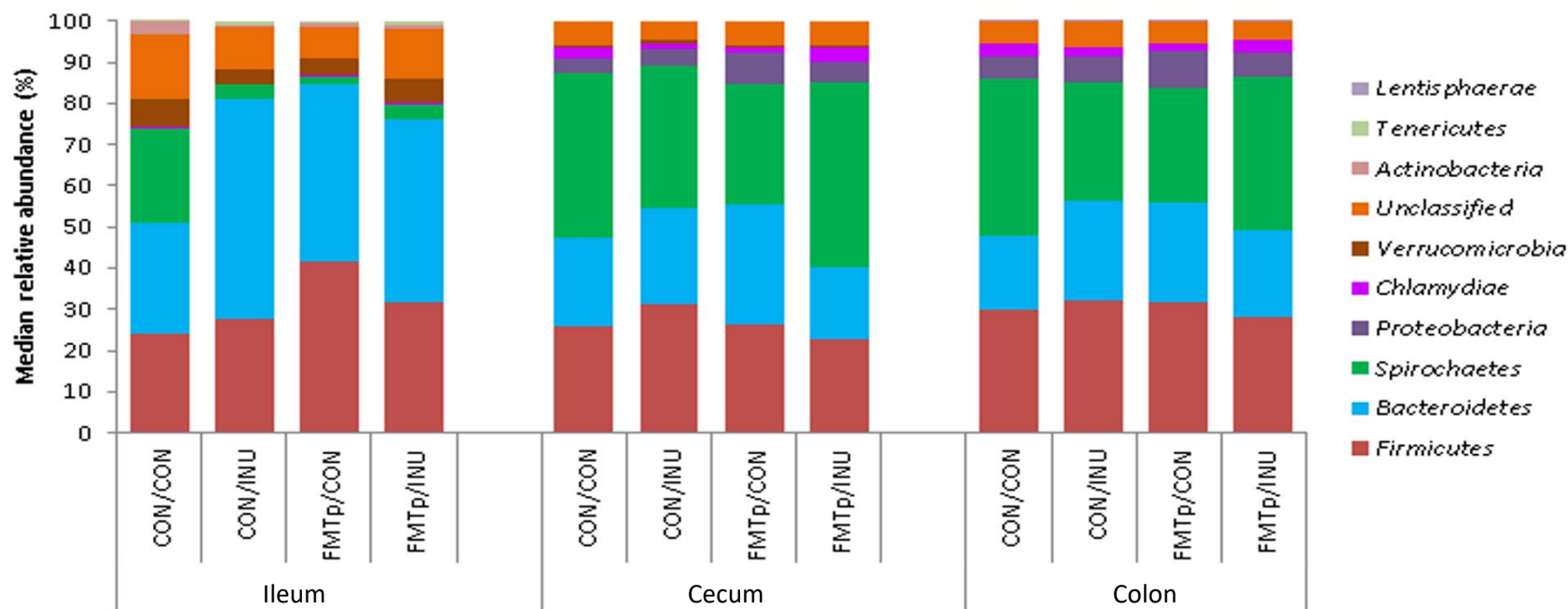
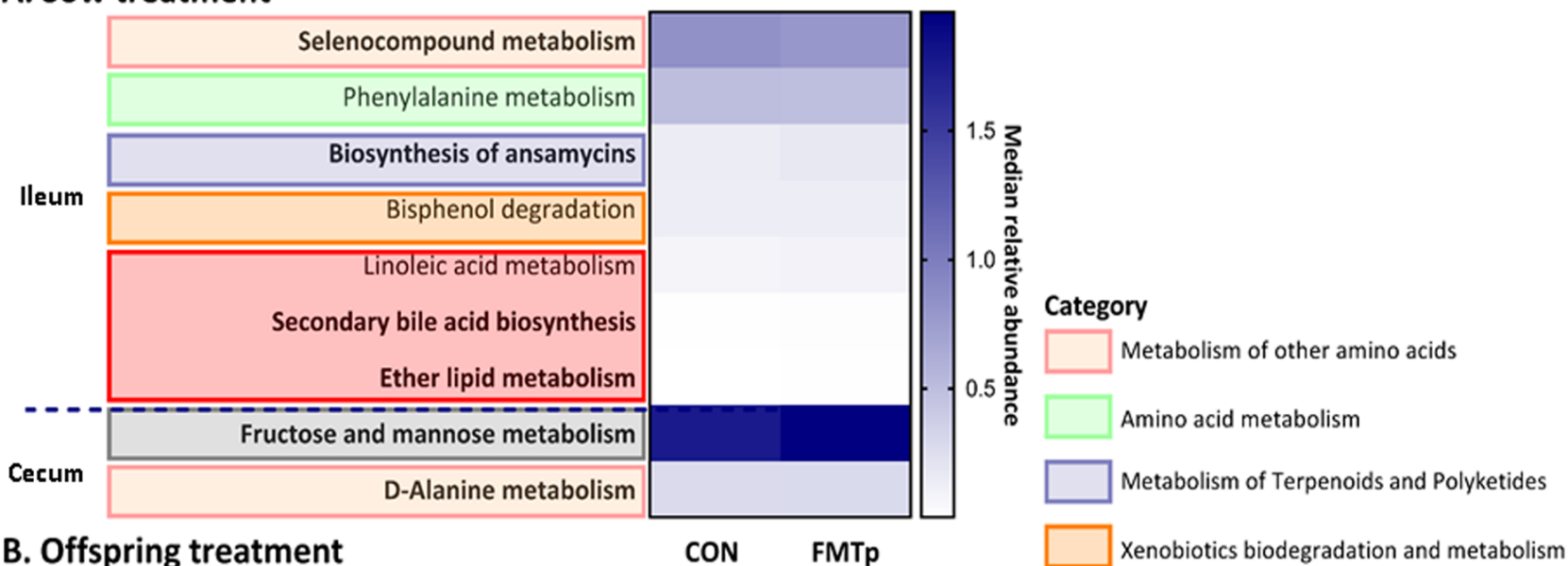


Fig S2. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on microbial composition at the phylum level in offspring in A. feces at 28, 50, 65, 100 and 130 days of age, and B. digesta from ileum, cecum and colon

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. Sow treatment



B. Offspring treatment

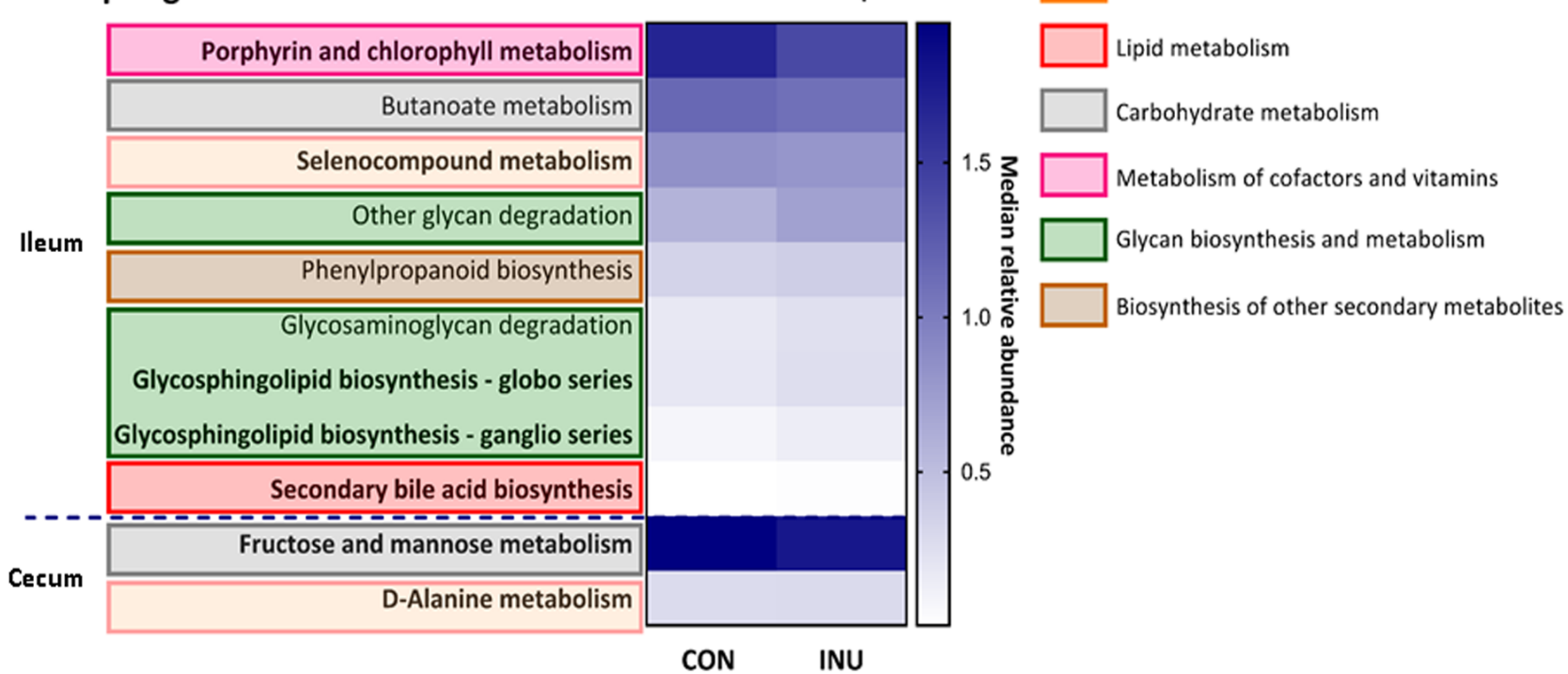


Fig. S3. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on predicted functional pathways for offspring fecal and intestinal microbiota at A. sow and B. offspring treatment level

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.

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.

Predicted bacterial pathways in bold depict those also affected by a sow x offspring treatment interaction. Additional sow treatment x offspring treatment interactions not shown in either panel A or B are shown in Table S1.

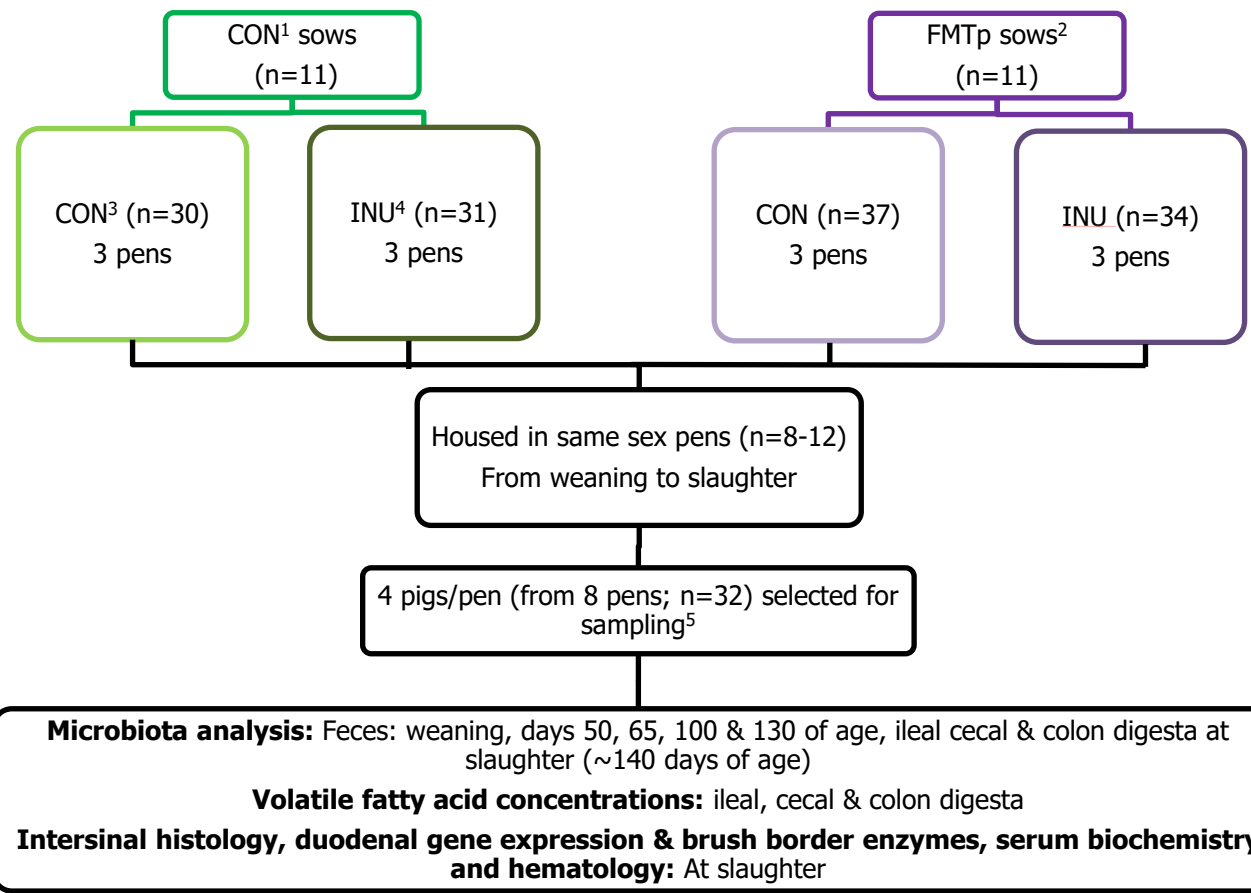


Fig S4. Schematic depicting sow treatments, offspring treatments and sample collection.

Sows: ¹CON: control; ²Fecal microbiota transplantation procedure (FMTp), 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: ³CON: control or ⁴INU: inulin for the first 6 weeks post-weaning (2% for the first 3 weeks, and 3% for the next 3 weeks). Thereafter, all pigs were fed the same finisher diet until slaughter at 140 days of age.

⁵32 pigs selected; Sow treatment level: CON: n=16; FMTp: n=16; Pig treatment level: CON: n=16; INU: n=16.