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Adding fermentable feed ingredients to pigs: changes or stability to the intestinal microbiota

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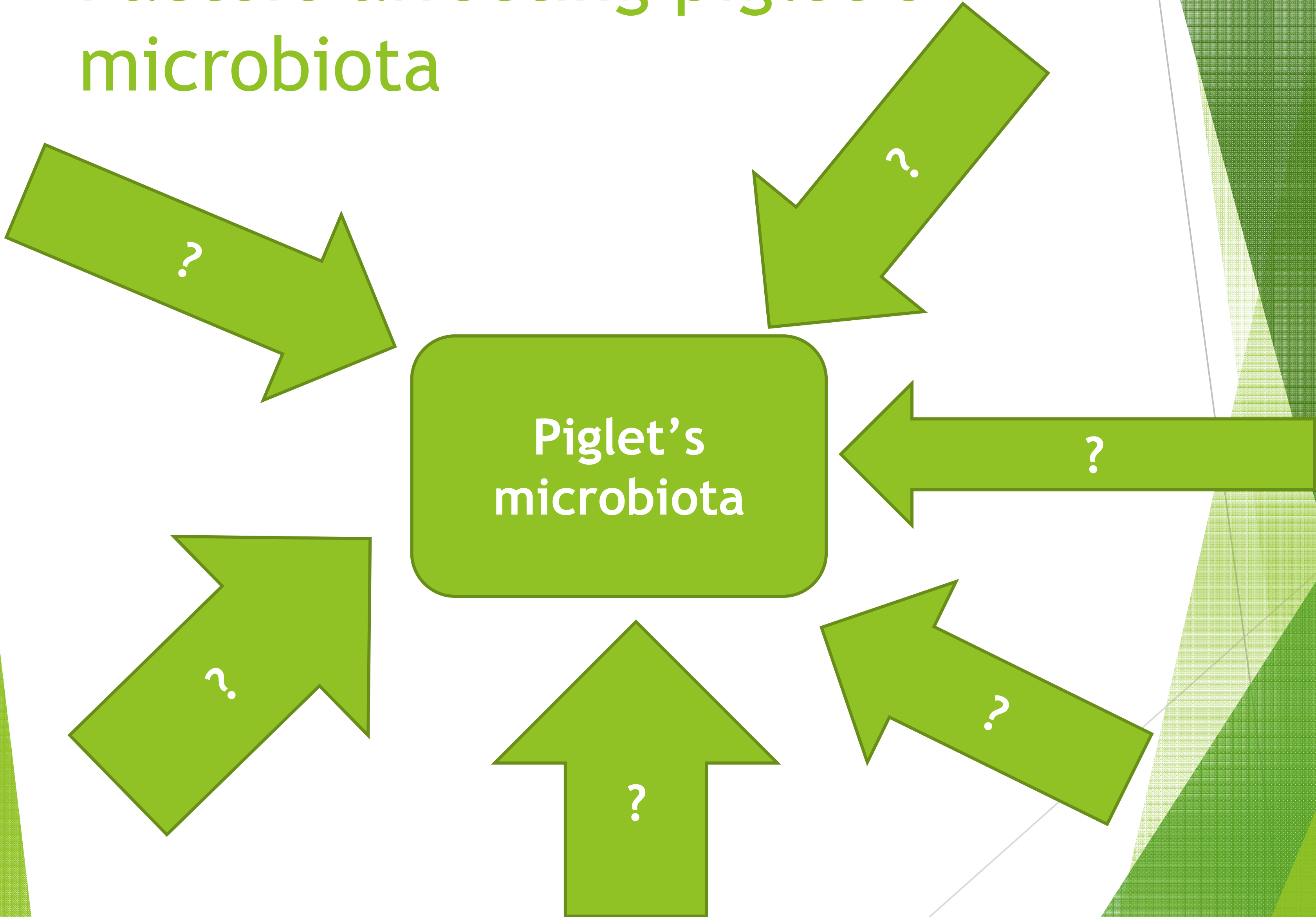
Factors affecting piglet's microbiota



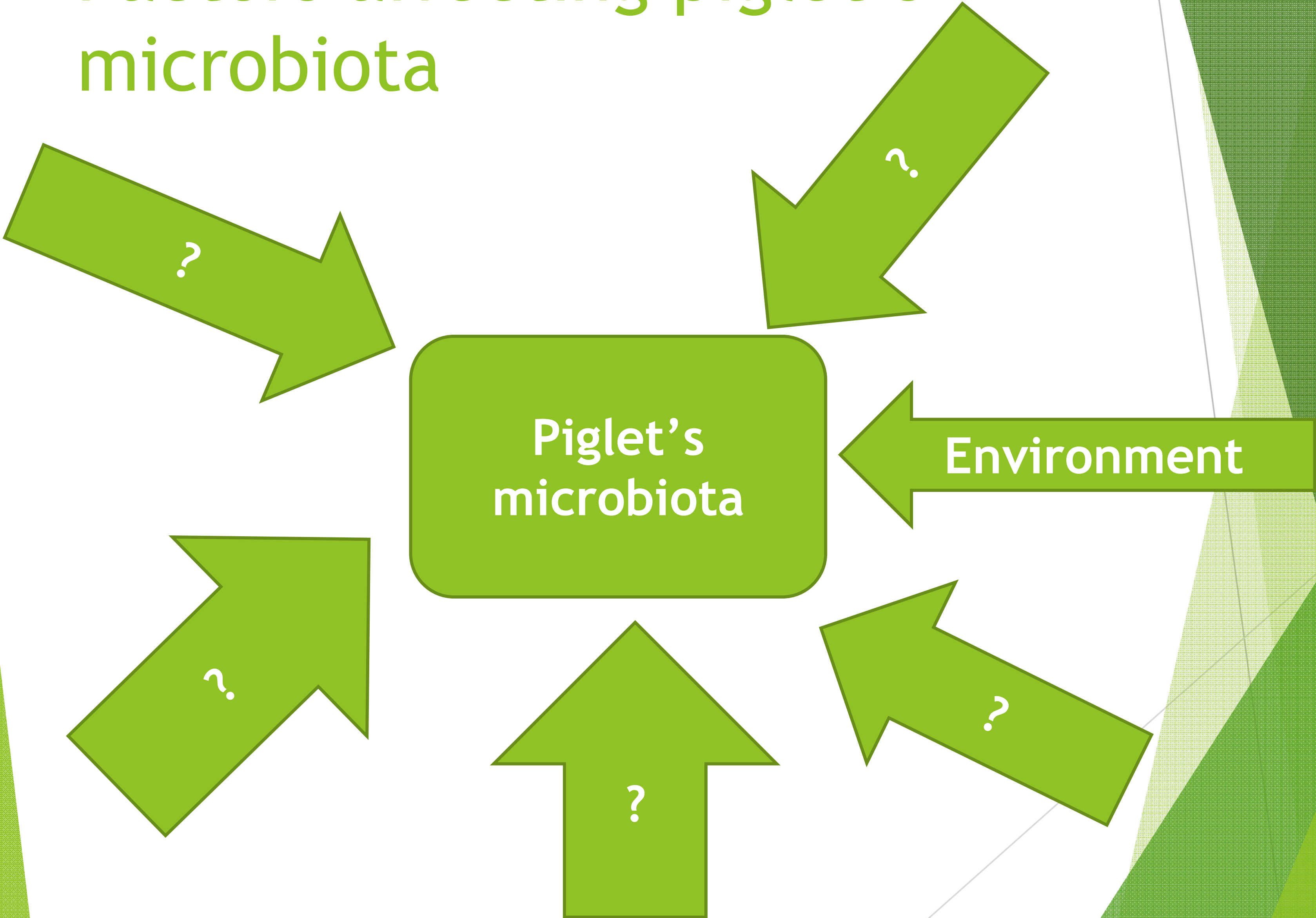
Early life colonization

- Pigs seperated from sow after birth and reared on milk replacer
- Non-siblings were co-housed in pairs
- The community of piglets **older than 31 days** was inferred to show **high stability relative to the first 28 days post birth**
- Significant **correlation** of microbial communities **between cohabiting piglets**, but not between siblings

Gut colonization in piglets is greatly influenced by the immediate environment

Thompson et al., 2008

Factors affecting piglet's microbiota



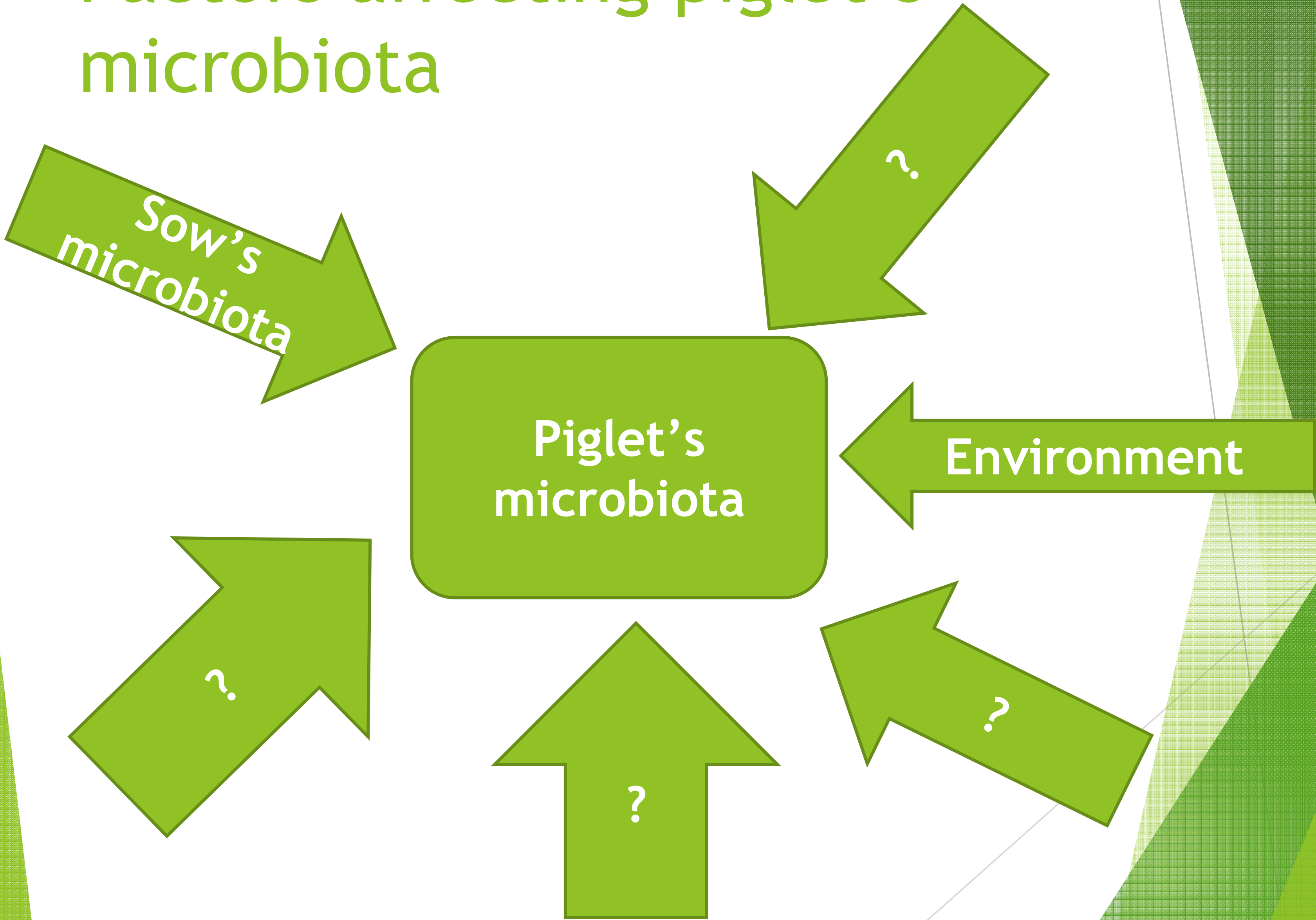
Early life colonization

- Piglets born by caesarian section, kept in clean isolators during 28 d
- Two treatments:
  - Inoculum + saline inoculations (SA)
  - Inoculum + complex sow's faeces' inoculations (CA)
- *Inoculum: Lactobacillus amylovorus*, *Clostridium glycolicum*, and *Parabacteroides* spp.
- Faecal microbiota composition **increased in diversity in time**
- **Clustering of CA faeces with sow's faeces**
- **SA treatments**: a **lower similarity** in microbial composition (diarrhea)

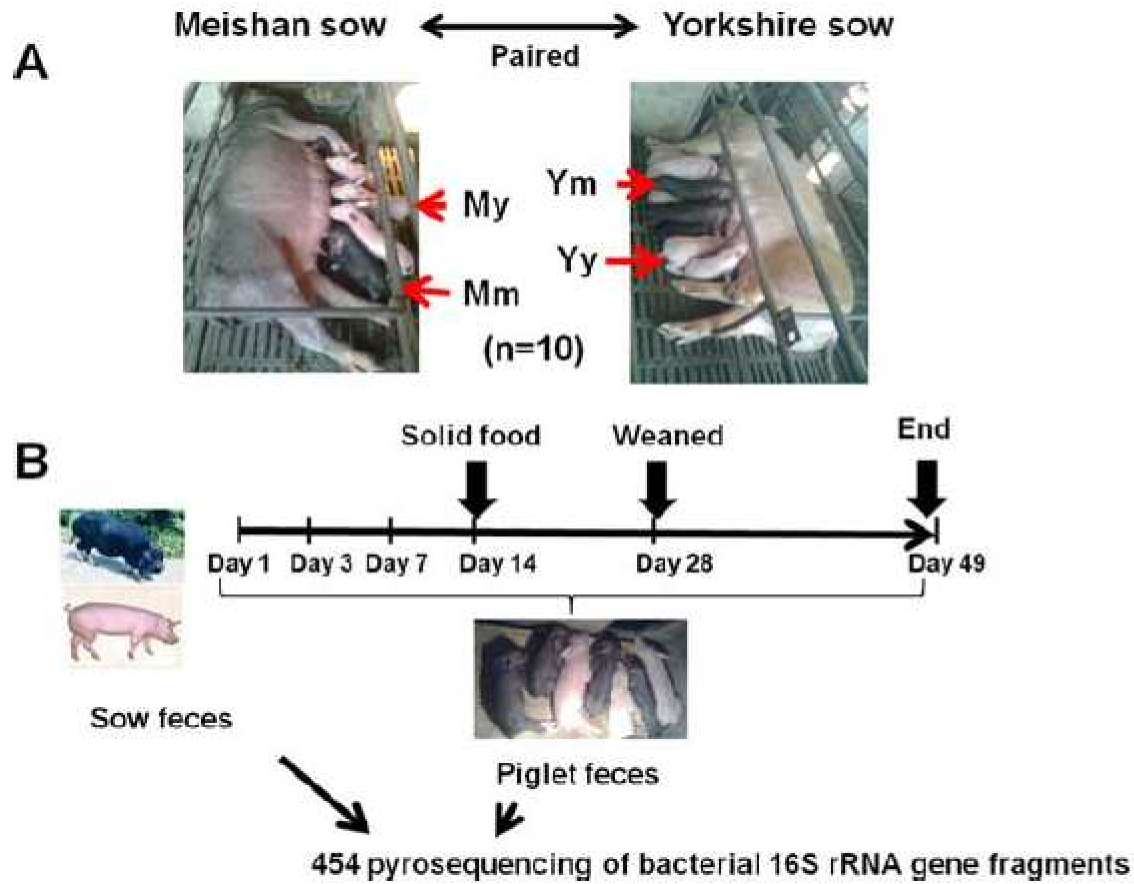
Microbiota from piglets is influenced by the sow's microbiota and by the environment

Jansman et al., 2012

Factors affecting piglet's microbiota

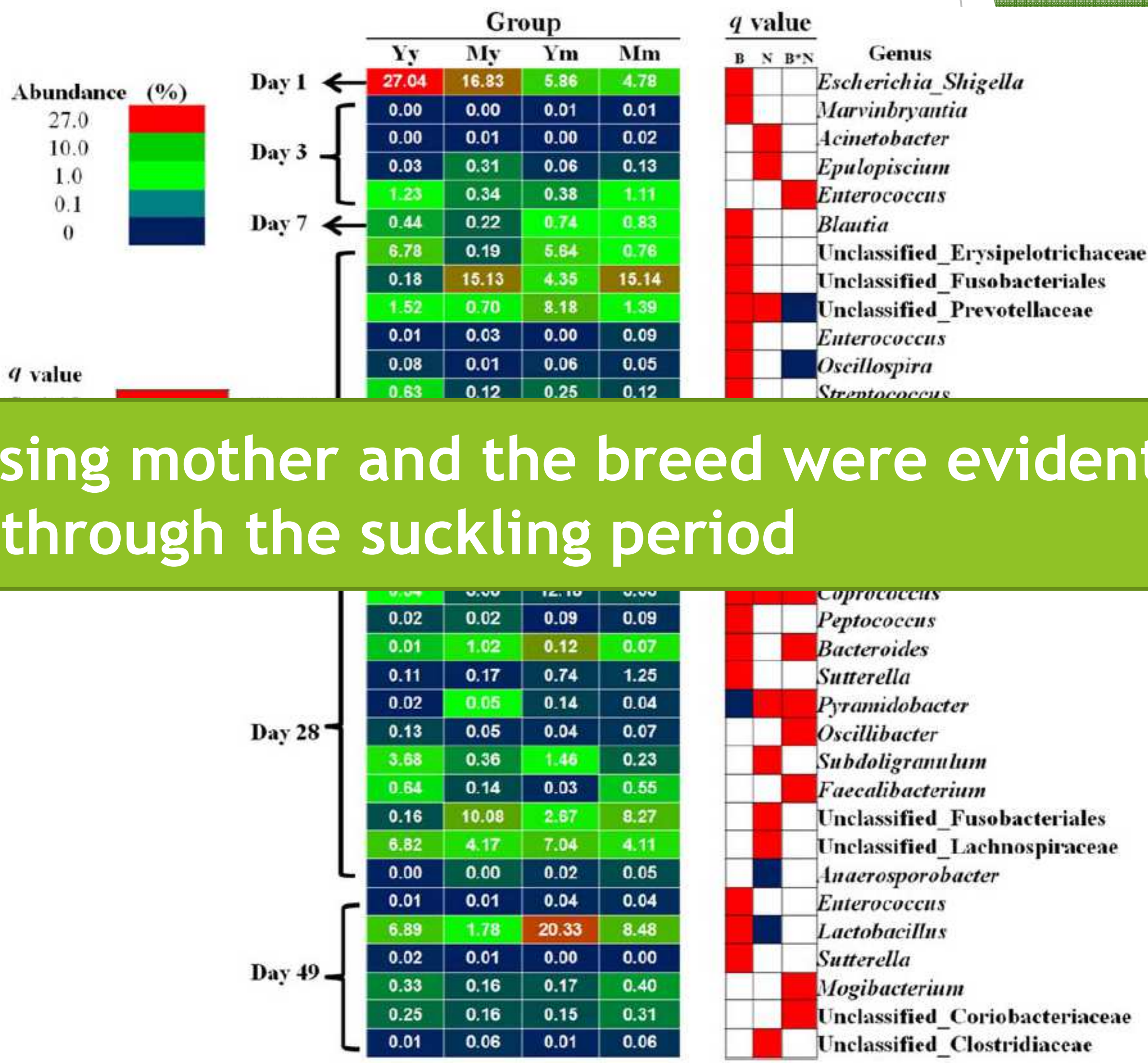


Study on the effect of breed or nursing mother



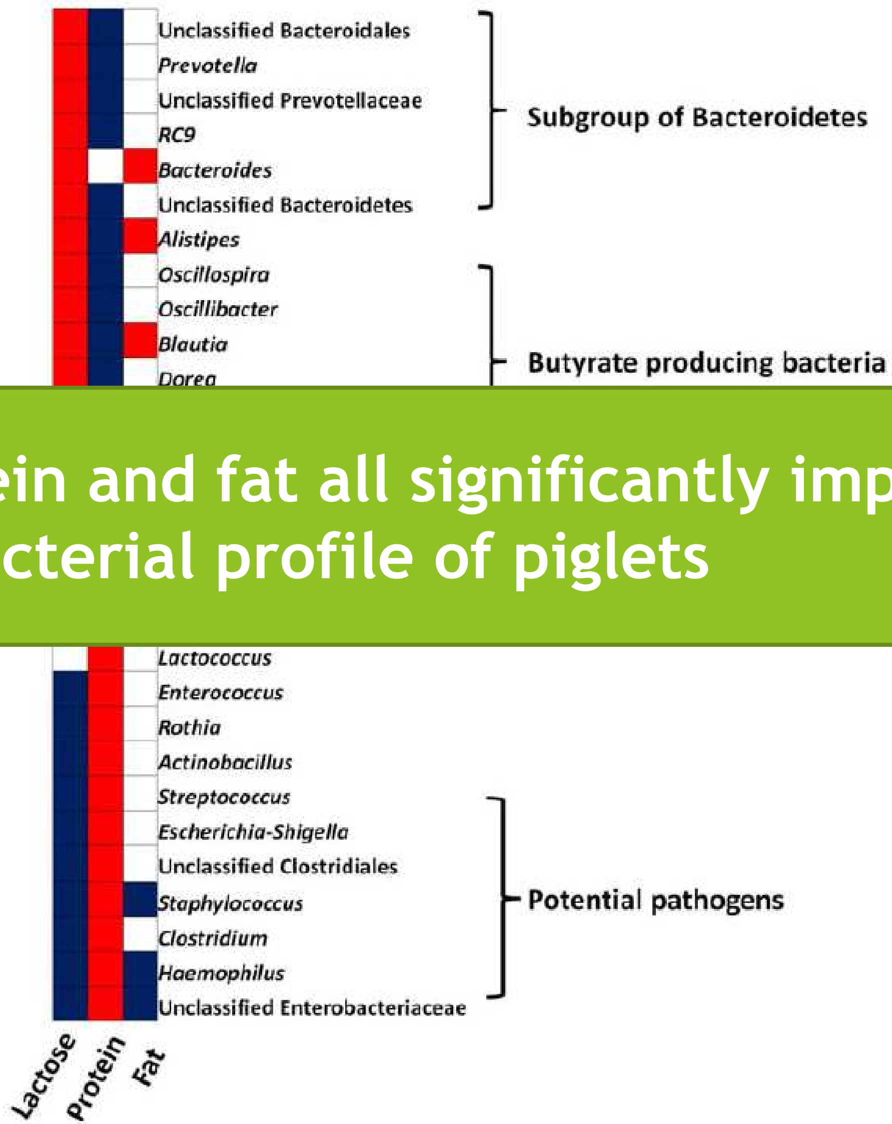
Bian et al., 2016

Effects of breed and nursing mother



Effect of nursing mother and the breed were evident through the suckling period

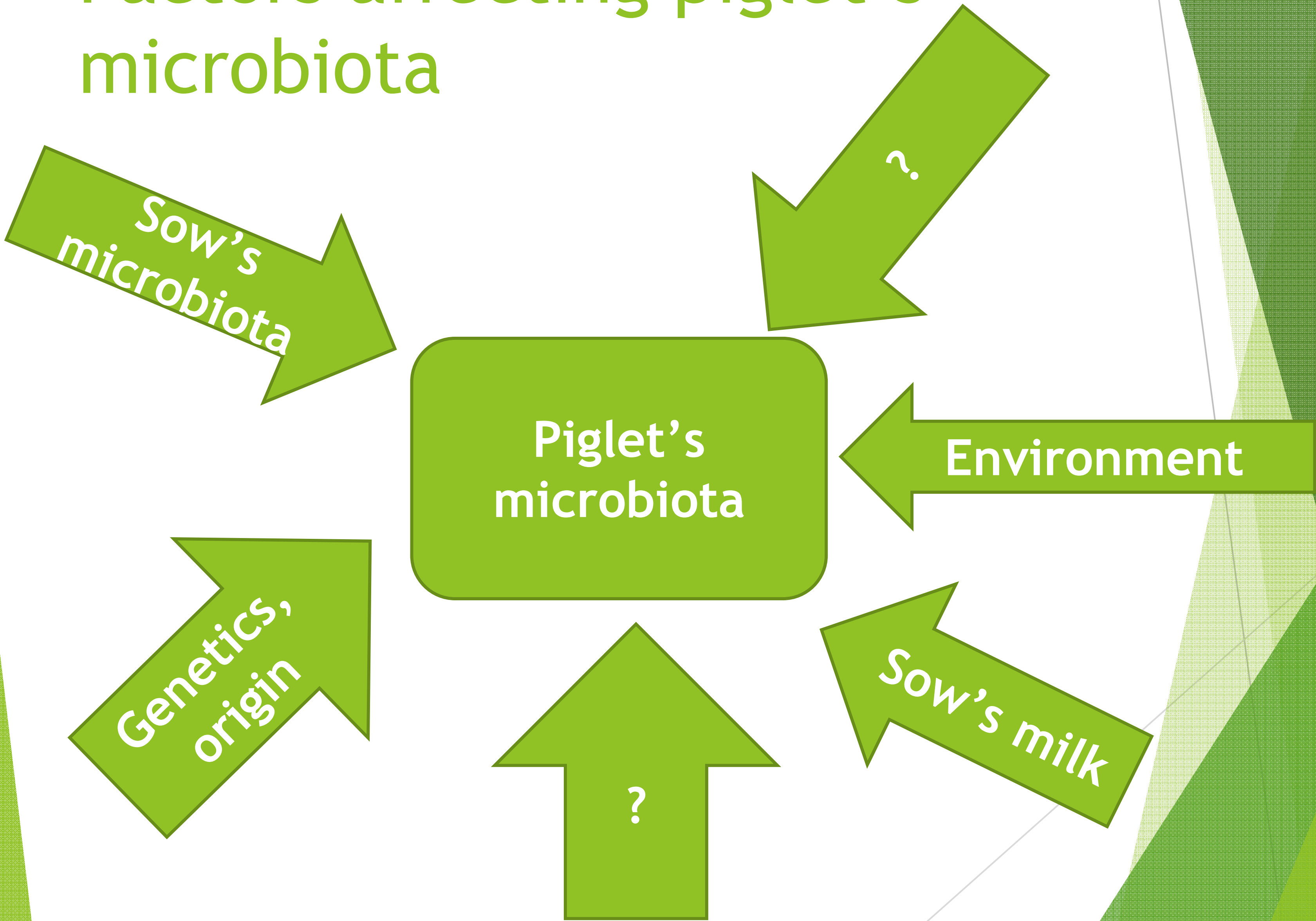
Impact of milk composition



Milk lactose, protein and fat all significantly impacted the bacterial profile of piglets

Red: positive correlation
Blue: negative correlation

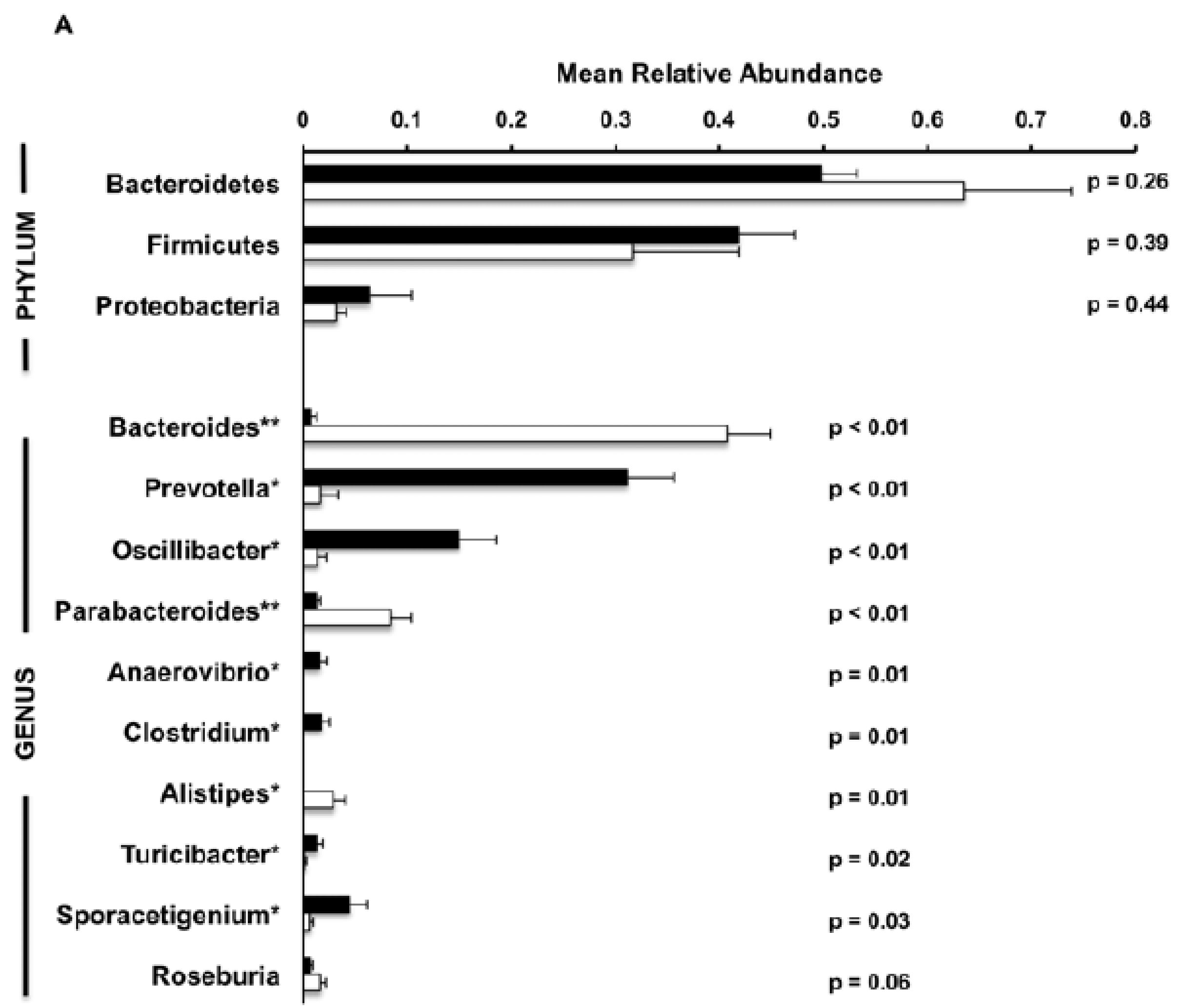
Factors affecting piglet's microbiota



Mother-fed vs formula milk

D21
Cecal content

Black: mother fed
White: formula fed



Poroyko et al., 2011

Effects of antibiotic or stress-treatment on d4

D8
Jejunal content

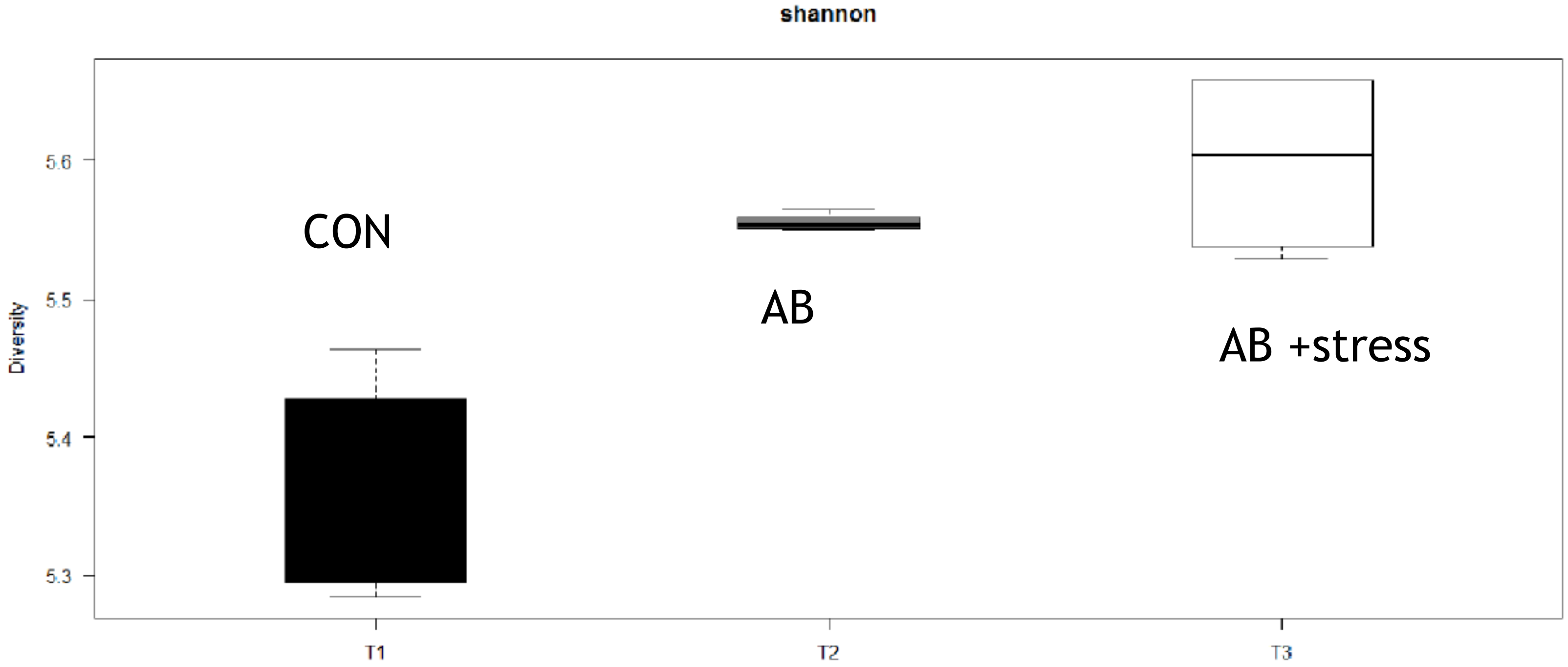


Figure 2. Diversity in microbiota in the three treatment groups. The Shannon index (y-axis) was calculated for all three treatments (T1, T2, and T3) (x-axis).
doi:10.1371/journal.pone.0100040.g002

Schokker et al., 2014

Impact on microbiota composition

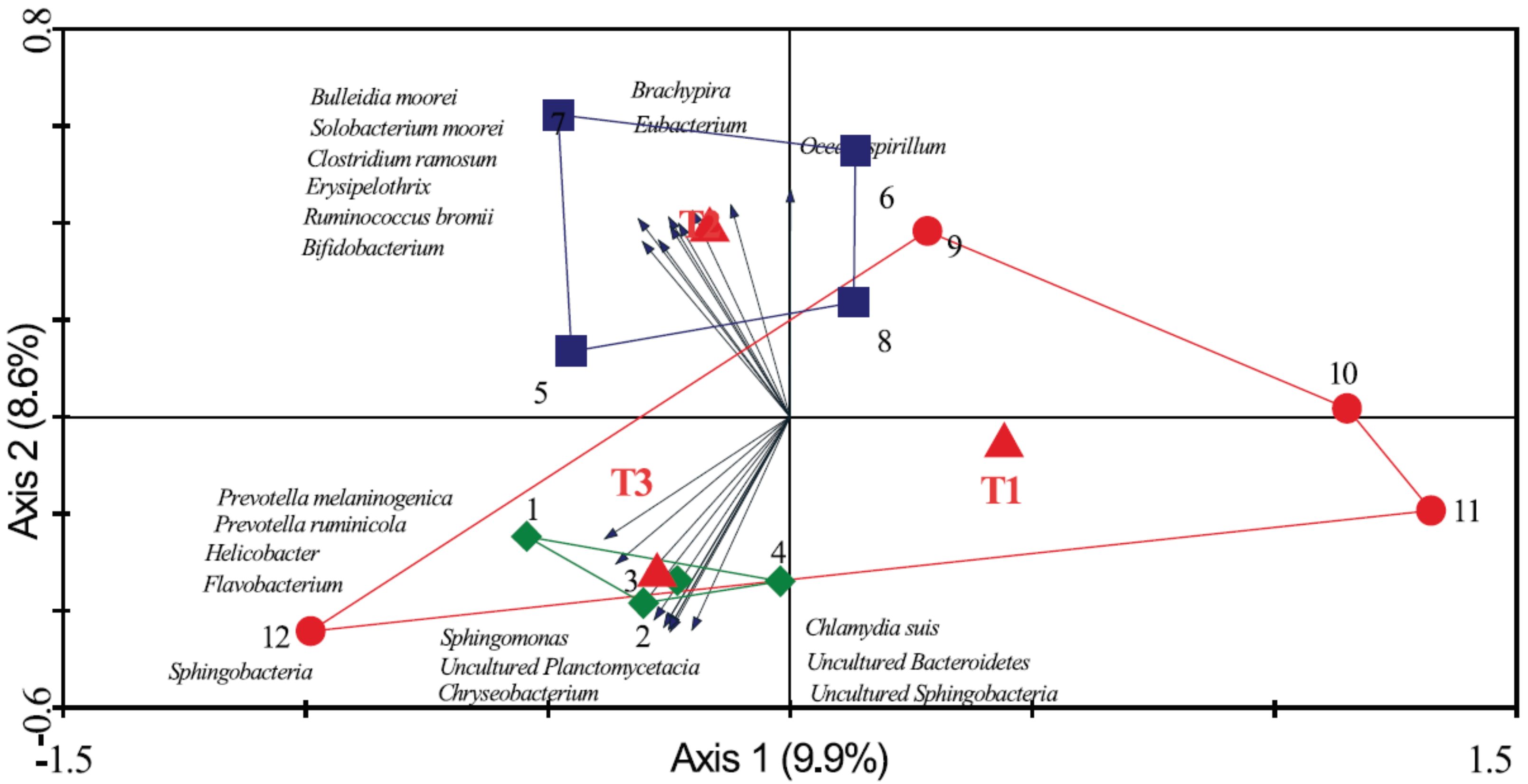


Figure 1. Triplot for RDA analysis of jejunal microbiota composition. Nominal environmental variables T1, T2 and T3 are represented by red triangles (▲). Samples are grouped by treatment: T1 (red; ○), T2 (blue; □) and T3 (green; ◇), each symbol represents a pool of four pigs, and numbers represent pool identity number. Microbial groups contributing at least 60% to the explanatory axes are represented as vectors. Both axes together explain 18.5% of the total variance in the dataset.

doi:10.1371/journal.pone.0100040.g001

Effects on transcriptome

- Differences in immune programming in early-life
- Antibiotic treatment versus control

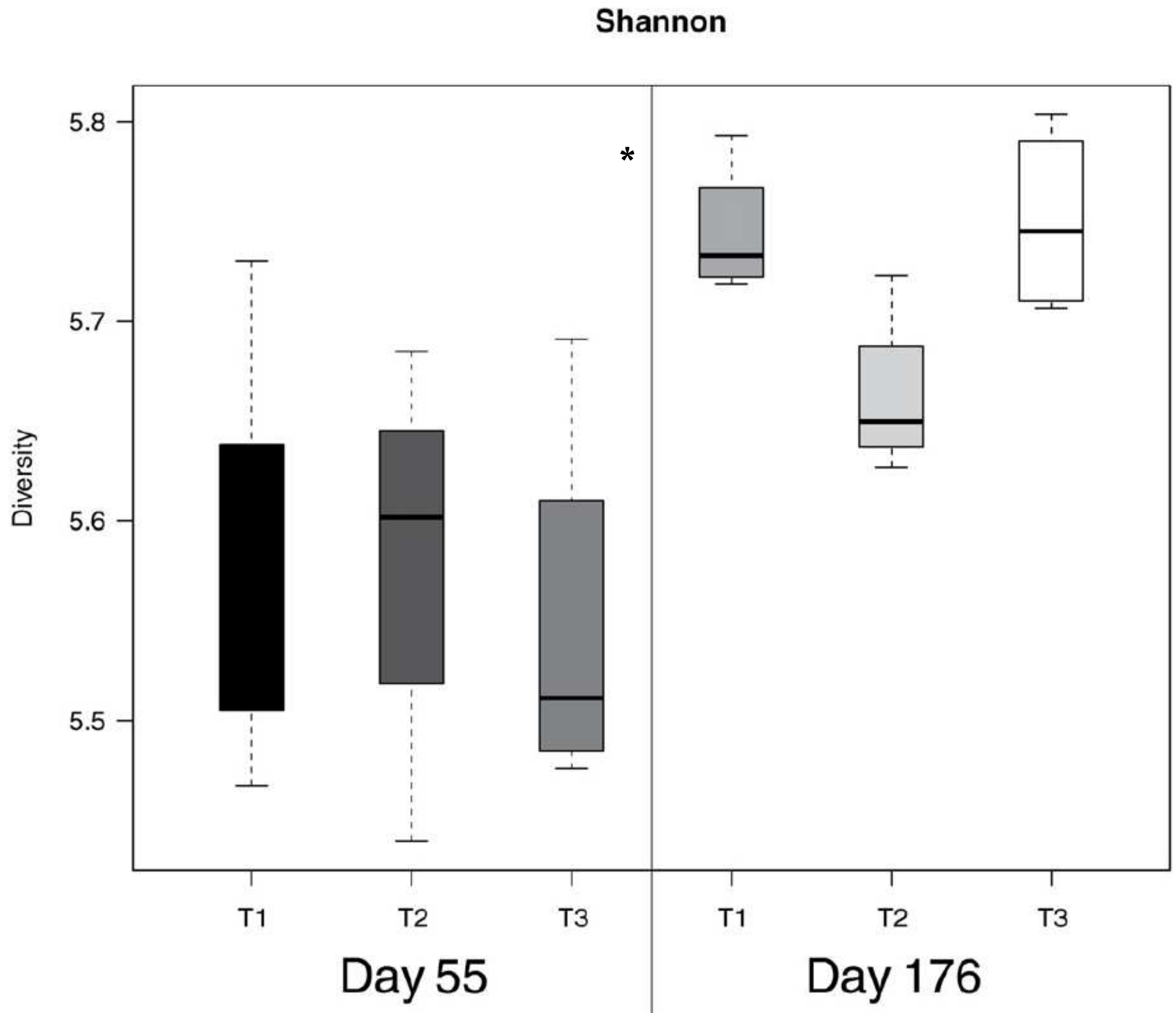
Table 2. Functional analysis of genes differentially expressed between treatment 2 versus 1.

	DOWN	
JEJUNUM	**#**	**Name**
	1	chemotaxis
	2	cytokine activity
	3	chemokine activity
	4	regulation of secretion/immune effector process
	5	cell migration/motion (leukocyte)
ILEUM	**#**	**Name**
	1	cytokine activity
	2	chemotaxis
	3	second-messenger-mediated signaling (cAMP)
	4	chemokine activity
	5	response to bacterium/regulation of systemic process

doi:10.1371/journal.pone.0100040.t002

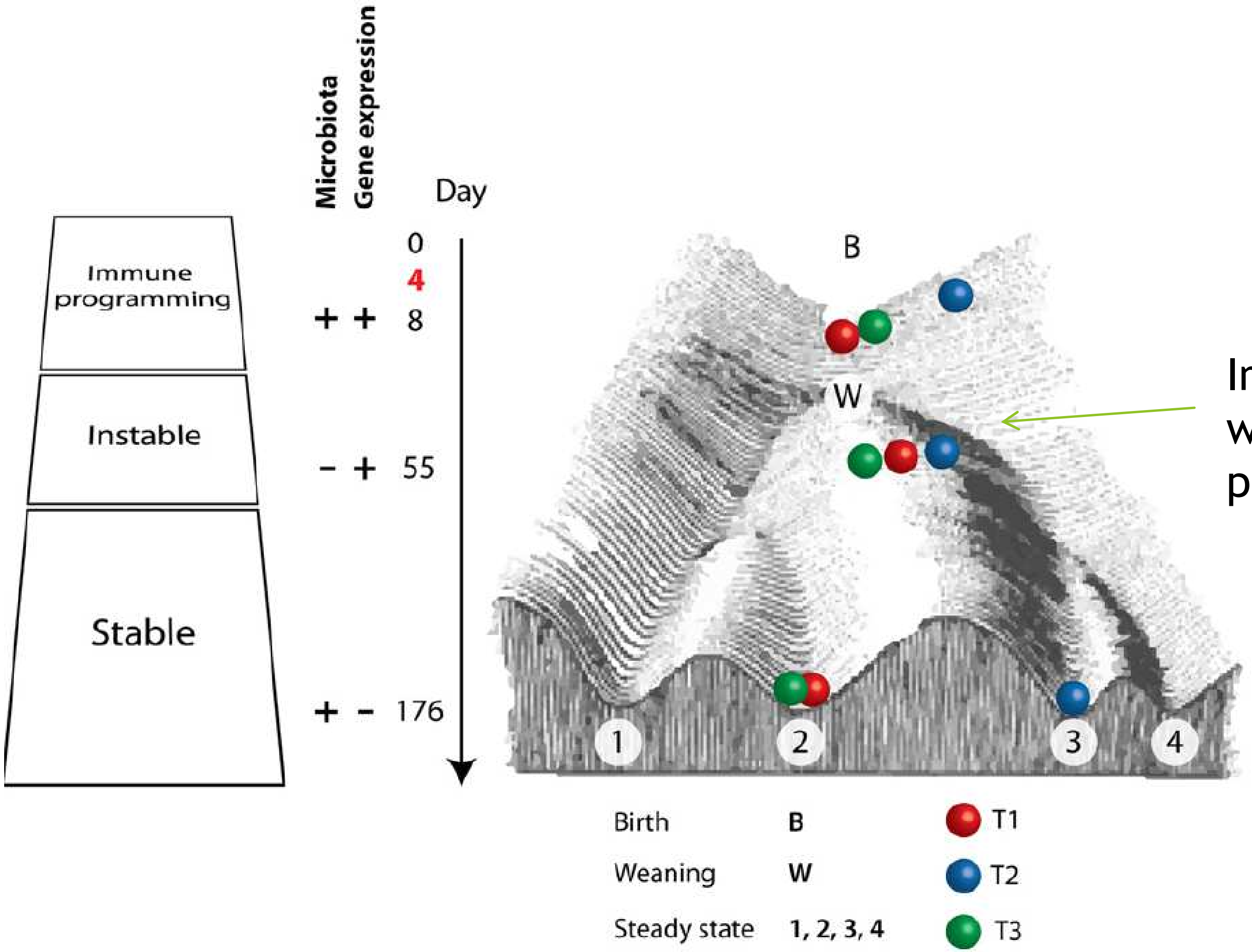
Long-lasting effects on microbiota

Jejunal content



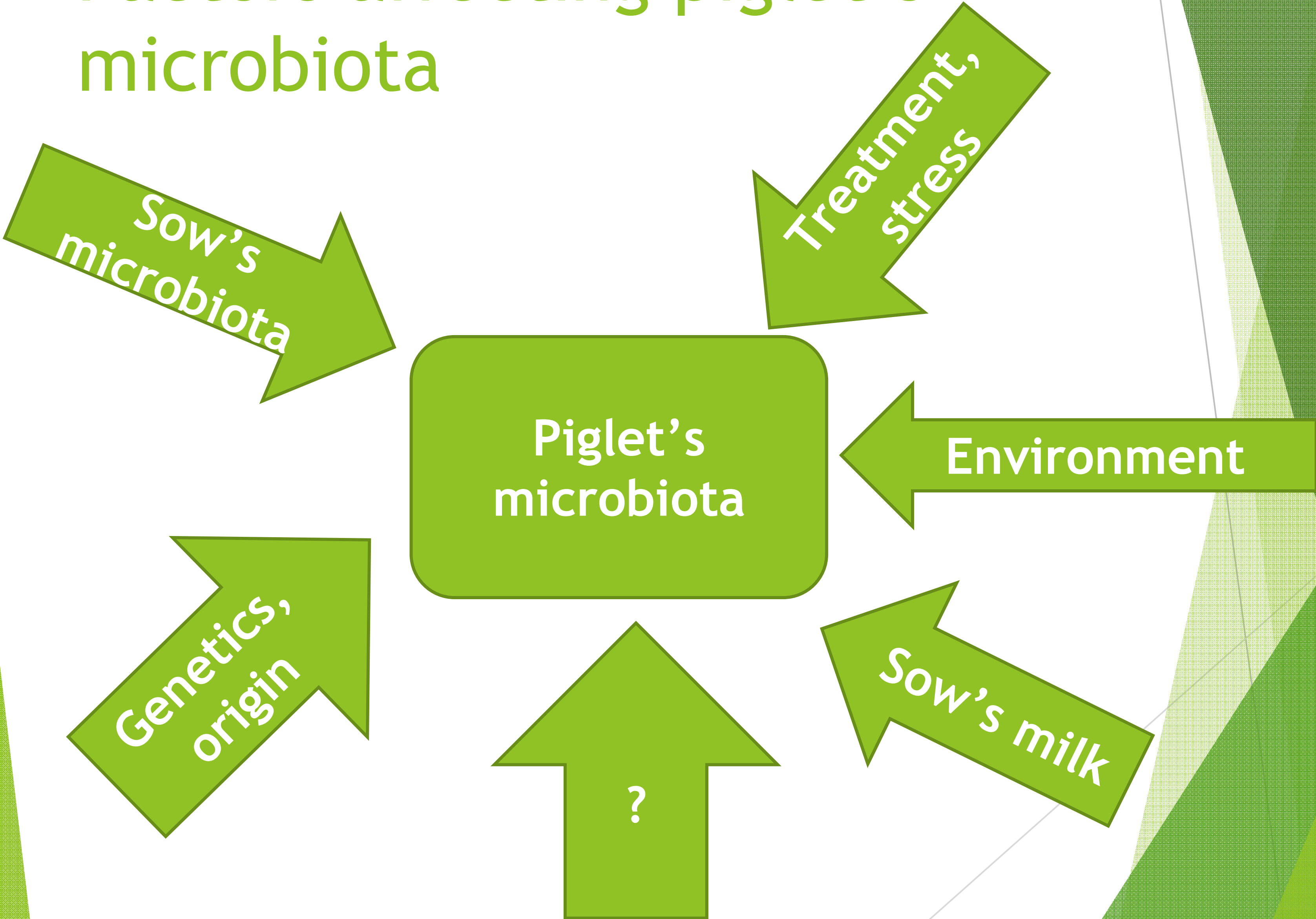
Schokker et al., 2015

Towards a differential gut homeostasis



Impact of weaning still present

Factors affecting piglet's microbiota



Corn starch vs Raw potato starch

From d70-d170

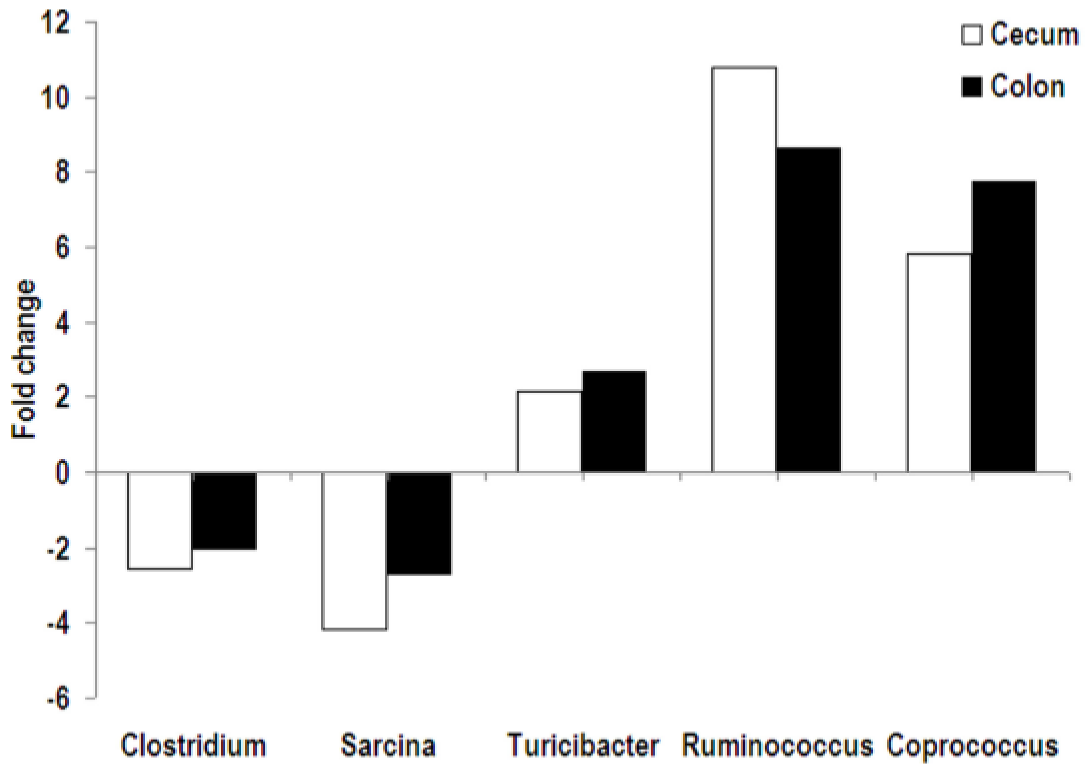


FIGURE 2 | Fold change of relative abundance (RPS vs. CS). Bacterial genera were significantly affected by dietary RPS in the cecum and colon of pigs.

Sun et al., 2016

Clustering according to intestinal segment and dietary treatment

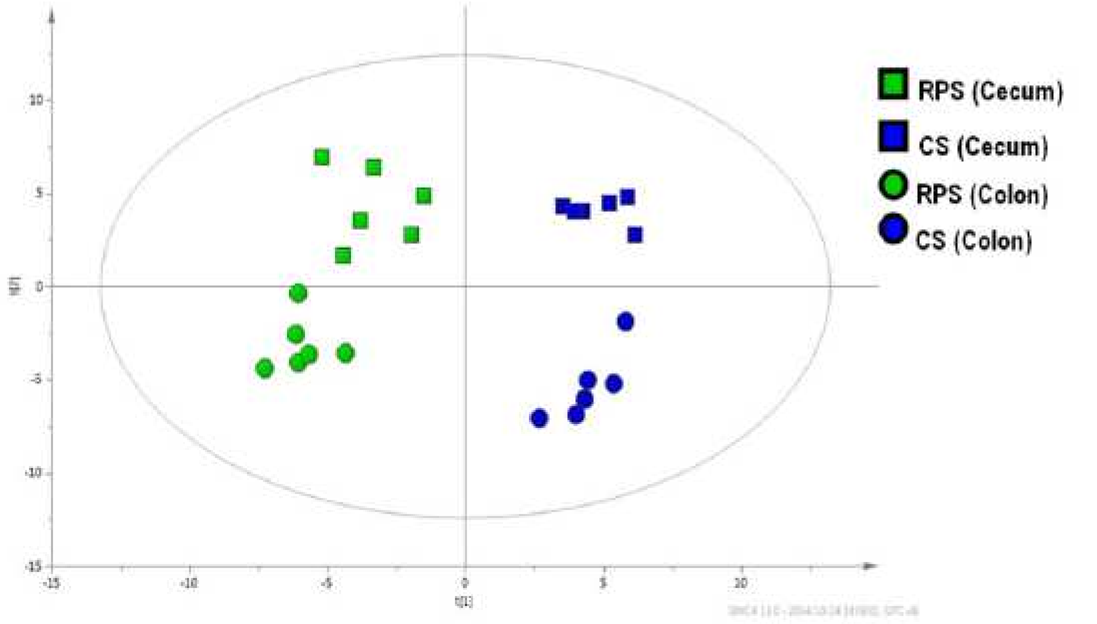
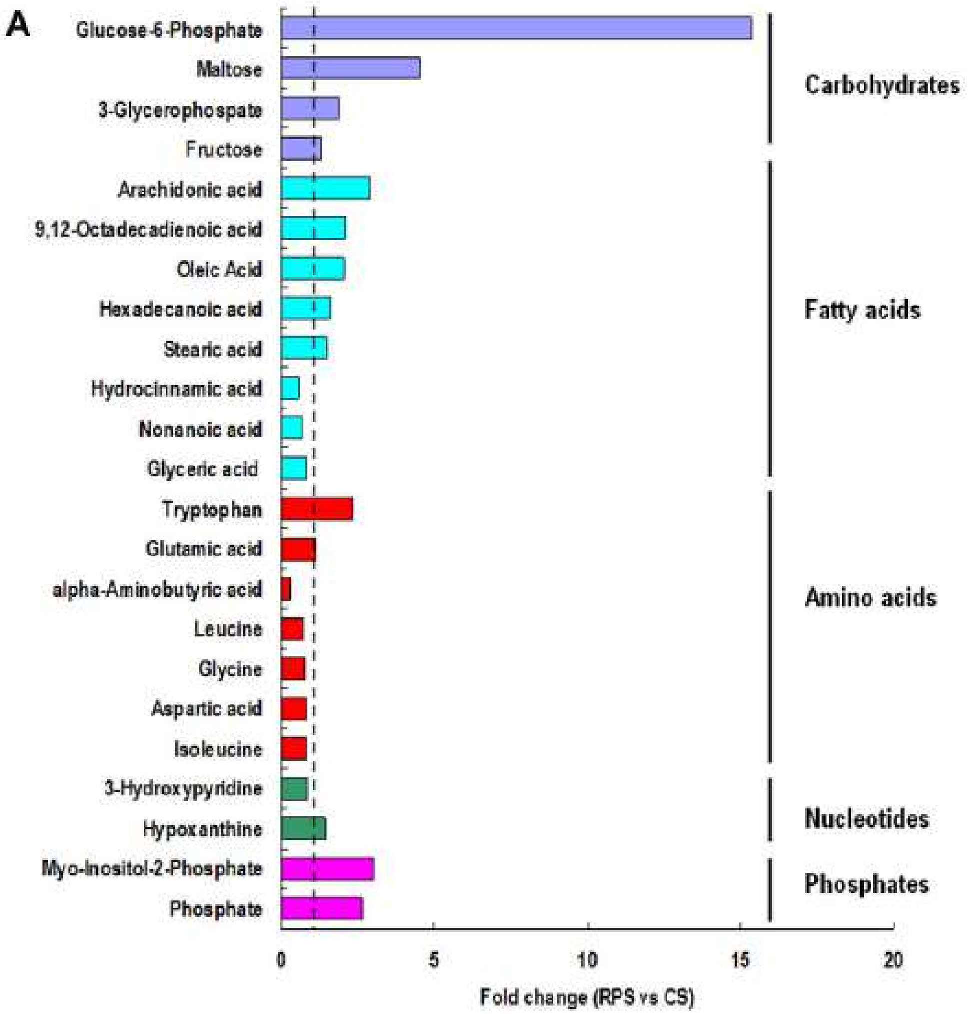
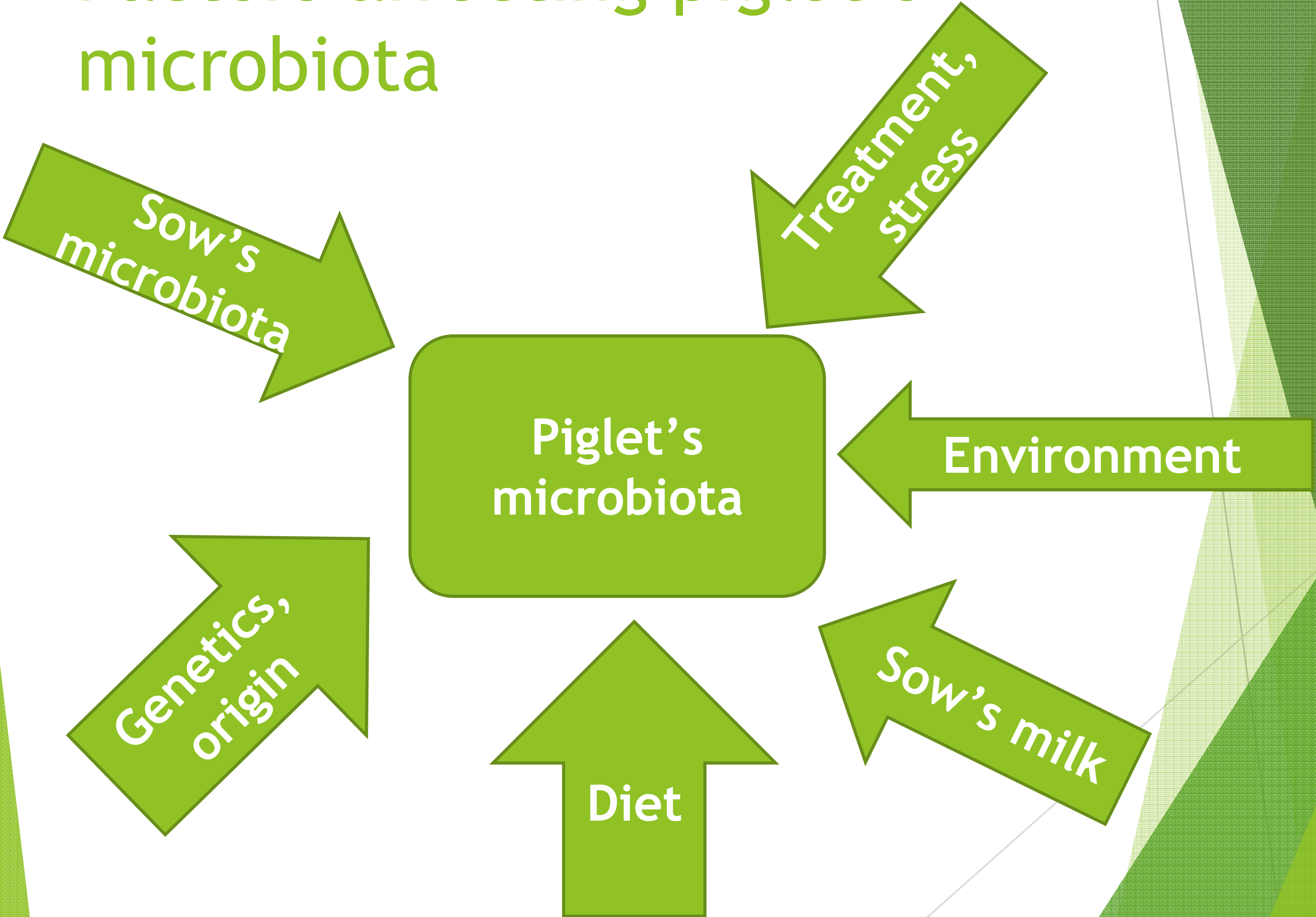


FIGURE 3 | Partial least squares discriminant analysis (PLS-DA).
PLS-DA of microbial metabolites in cecal and colonic contents from pigs fed corn starch (CS) and raw potato starch (RPS).

Resulting in different metabolites produced



Factors affecting piglet's microbiota



Adding fermentable feed ingredients to pigs: changes or stability to the intestinal microbiota

AIM

- Reduce the risk of infections at weaning

STRATEGY

- Through action on microbiota

- Indirectly: Via sow
- Directly: Interventions on piglets

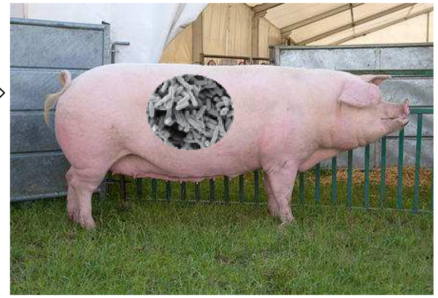
Effects by the maternal diet

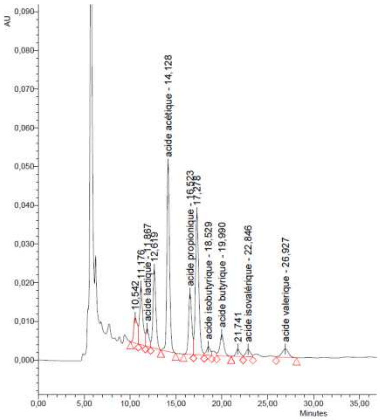
Inulin

Wheat bran

Resistant starch

Hypotheses





AU
0,090
0,080
0,070
0,060
0,050
0,040
0,030
0,020
0,010
0,000
10,542
11,176
acide lactique - 11,867
12,619
acide acétique - 14,128
acide propionique - 16,523
17,278
acide isobutyrique - 18,529
acide butyrique - 19,990
21,741
acide isovalérique - 22,846
acide valerique - 26,927
0,00
5,00
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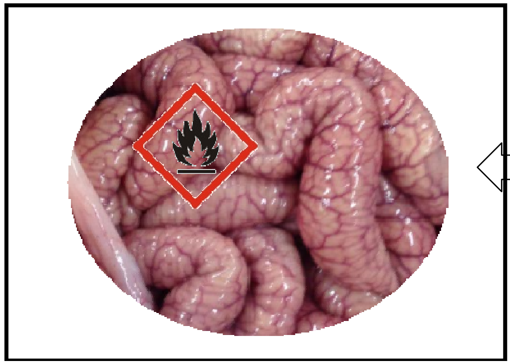
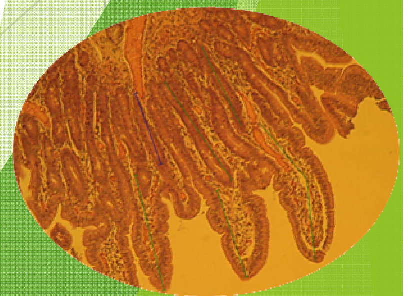
Minutes





Inulin in the diet of gestating and lactating sows

- 3% inulin diet from 14d a.p.;
- change from gestation to lactation diet on 1d p.p.

Table 1 Microbial cell counts (log_{10}/g wet weight) in the faeces of sows fed a diet without (C) or with inulin (I)

	Day 4 a.p.		Day 1 p.p.		Day 5 p.p.		*P*-value		
	C (n[1] = 10)	I (n = 10)	C (n = 10)	I (n = 9)	C (n = 10)	I (n = 10)	Diet	Time	Diet*time
Eubacteria	10.6 ± 0.51	10.8 ± 0.85	9.86 ± 0.10	9.75 ± 0.21	10.8 ± 0.15	10.8 ± 0.18	0.847	**0.005**	0.718
Enterobacteria	7.21 ± 1.01	6.66 ± 0.90	7.16 ± 0.98	7.77 ± 0.75	7.42 ± 0.68	7.54 ± 0.99	0.802	0.103	**0.017**
Enterococci	6.42 ± 0.45	6.83 ± 0.59	5.96 ± 0.31	7.00 ± 0.91	6.62 ± 0.48	6.98 ± 0.61	**0.014**	0.109	**0.028**
Bifidobacteria	7.92 ± 1.05	8.10 ± 1.91	7.24 ± 0.51	7.60 ± 1.08	8.05 ± 0.87	7.92 ± 1.51	0.976	0.052	0.357
Lactobacilli	9.39 ± 0.62	9.32 ± 0.77	8.89 ± 0.67	7.73 ± 1.42	8.98 ± 0.57	8.60 ± 0.89	0.109	**<0.001**	0.051
L. reuteri	8.37 ± 0.86	8.25 ± 0.64	7.36 ± 1.12	6.83 ± 0.66	7.93 ± 0.73	7.57 ± 0.47	0.305	**<0.001**	0.244
L. amylovorus	9.39 ± 0.40	9.32 ± 0.35	8.50 ± 0.96	8.22 ± 0.63	9.12 ± 0.61	9.03 ± 0.32	0.502	**<0.001**	0.606
L. johnsonii	6.16 ± 1.10	5.81 ± 0.94	4.97 ± 0.79	5.20 ± 0.52	5.46 ± 0.42	5.25 ± 0.48	0.872	**0.002**	0.411
L. mucosae	8.22 ± 0.83	7.83 ± 0.41	7.08 ± 0.89	6.72 ± 0.47	7.56 ± 0.61	7.17 ± 0.96	0.088	**<0.001**	0.898
C. leptum	10.5 ± 0.24	10.1 ± 0.55	9.50 ± 0.97	9.84 ± 0.62	9.89 ± 0.73	9.91 ± 1.11	0.987	**0.010**	0.266
C. coccoides	10.7 ± 0.53	10.8 ± 0.24	9.69 ± 1.05	9.79 ± 0.77	10.6 ± 0.41	10.5 ± 0.93	0.891	**0.002**	0.762
BPP	9.66 ± 0.53	9.69 ± 0.54	9.40 ± 0.25	9.31 ± 0.37	9.83 ± 0.52	9.55 ± 0.28	0.152	0.052	0.557

Paβlack et al., 2015

Effects on the microbial metabolites of sows

- Change in microbial metabolites

Table 2 Microbial metabolites and pH in the faeces of sows fed a diet without (C) or with inulin (I)

	Day 4 a.p.		Day 1 p.p.		Day 5 p.p.		*P*-value		
	C (n = 11)	I (n = 10)	C (n = 11)	I (n = 9)	C (n = 11)	I (n = 10)	Diet	Time	Diet*time
pH	6.79 ± 0.23	6.63 ± 0.34	6.99 ± 0.34	6.59 ± 0.36	6.68 ± 0.12	6.63 ± 0.23	**0.007**	0.323	0.167
L-lactate (mmol/kg)	0.73 ± 0.55	0.70 ± 0.30	0.43 ± 0.28	0.28 ± 0.22	0.35 ± 0.18	0.24 ± 0.19	0.185	**0.002**	0.909
D-lactate (mmol/kg)	0.62 ± 0.64	0.54 ± 0.26	0.20 ± 0.20	0.14 ± 0.12	0.16 ± 0.12	0.11 ± 0.11	0.451	**0.002**	0.947
Ammonia (mmol/kg)	32.6 ± 19.5	47.0 ± 29.1	13.7 ± 9.64	20.2 ± 10.4	21.7 ± 10.2	27.3 ± 12.9	0.070	**0.001**	0.586
SCFA (mmol/l)	152 ± 44.4	155 ± 30.1	100 ± 31.4	123 ± 31.5	138 ± 38.3	158 ± 17.5	0.164	**<0.001**	0.602
Acetic acid (mol. %)	54.4 ± 3.61	53.7 ± 3.78	60.6 ± 4.11	56.8 ± 4.19	60.9 ± 4.35	59.1 ± 3.16	0.081	**<0.001**	0.388
Propionic acid (mol. %)	23.0 ± 1.08	22.6 ± 1.33	19.3 ± 2.03	20.9 ± 1.11	19.4 ± 1.40	19.9 ± 1.69	0.153	**<0.001**	0.053
i-butyric acid (mol. %)	2.87 ± 0.56	2.79 ± 0.33	2.87 ± 0.40	2.61 ± 0.55	2.52 ± 0.27	2.51 ± 0.32	0.337	**0.037**	0.641
n-butyric acid (mol.%)	13.0 ± 2.85	13.9 ± 2.62	10.4 ± 4.24	12.8 ± 3.36	11.1 ± 3.54	12.4 ± 1.71	0.152	**0.025**	0.711
i-valeric acid (mol. %)	4.05 ± 0.91	3.92 ± 0.51	4.06 ± 0.62	3.88 ± 0.86	3.62 ± 0.43	3.56 ± 0.53	0.538	0.068	0.950
n-valeric acid (mol. %)	2.66 ± 0.50	3.09 ± 0.70	2.77 ± 0.51	3.02 ± 0.63	2.45 ± 0.51	2.61 ± 0.45	0.147	**0.012**	0.467

Abbreviations: a.p.: *ante partum*; p.p.: *post partum*; *SCFA* short chain fatty acids.
Boldface *P*-values indicate significant effects ($P \le 0.05$).

Altering the microbiota and metabolites of the piglets

Table 3 Microbial cell counts (log_{10}/g wet weight) in the digesta of the stomach and caecum of suckling piglets, where the mother sows received either a diet without (C) or with inulin (I)

	Stomach		Caecum	
	C ($n^1 = 7$)	I (n = 8)	C ($n^2 = 8$)	I (n = 8)
Eubacteria	10.3 ± 0.26[a]	11.0 ± 0.25[b]	11.7 ± 0.37	11.7 ± 0.33
Enterobacteria	4.56 ± 0.87[a]	3.45 ± 0.50[b]	9.02 ± 0.66	8.58 ± 1.20
Enterococci	7.24 ± 0.45	7.21 ± 0.74	6.76 ± 0.49[a]	7.57 ± 0.46[b]
Bifidobacteria	3.89 ± 0.70	5.04 ± 1.83	5.16 ± 0.91	5.57 ± 1.29
Lactobacilli	9.31 ± 0.88	9.06 ± 0.97	10.0 ± 1.23	9.94 ± 1.49
L. reuteri	8.23 ± 0.63	7.81 ± 0.84	8.40 ± 0.50	8.13 ± 0.62
L. amylovorus	9.28 ± 0.32[a]	8.17 ± 0.66[b]	9.26 ± 0.69	9.28 ± 0.79
L. johnsonii	7.05 ± 0.69	6.45 ± 0.93	6.76 ± 0.79	6.22 ± 1.09
L. mucosae	7.61 ± 0.82	6.78 ± 1.42	7.95 ± 0.89	7.97 ± 1.31
C. leptum	6.70 ± 0.31	6.40 ± 0.89	10.0 ± 0.78[a]	10.8 ± 0.15[b]
C. coccoides	7.02 ± 0.93	6.66 ± 1.02	10.3 ± 0.78	10.7 ± 0.43
BPP	6.14 ± 1.12	5.43 ± 1.72	9.87 ± 0.74	10.3 ± 0.32

On day 10

Altering the microbiota and metabolites of the piglets

Table 4 Microbial metabolites and pH in the digesta of suckling piglets, where the mother sows received a diet without (C) or with inulin (I)

	Stomach		Small intestine		Caecum		Rectum	
	C (n[1] = 8)	I (n = 8)	C (n[2] = 8)	I (n = 8)	C (n[3] = 8)	I (n = 8)	C (n[4] = 8)	I (n = 8)
pH	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Acetic acid (mol.%)	3.33 ± 1.75	3.52 ± 1.93	4.40 ± 2.08	3.28 ± 3.41	36.5 ± 9.78	33.9 ± 11.4	13.9 ± 6.75	9.68 ± 4.01
Propionic acid (mol.%)	0.12 ± 0.10	0.12 ± 0.20	0.31 ± 0.35	0.19 ± 0.30	10.8 ± 2.59	8.93 ± 3.26	3.83 ± 2.33	2.80 ± 2.36
i-butyric acid (mol.%)	0.01 ± 0.00[a]	0.01 ± 0.02[b]	0.02 ± 0.02	*	1.24 ± 0.49	0.90 ± 0.40	0.69 ± 0.48	0.47 ± 0.36
n-butyric acid (mol.%)	0.18 ± 0.04[a]	0.06 ± 0.07[b]	0.12 ± 0.11	0.11 ± 0.11	3.74 ± 1.68	3.10 ± 1.50	2.07 ± 1.61	1.21 ± 1.12
i-valeric acid (mol.%)	0.27 ± 0.52[a]	0.03 ± 0.03[b]	0.04 ± 0.03	0.03 ± 0.03	1.62 ± 0.56	1.13 ± 0.41	1.37 ± 0.87	0.86 ± 0.67
n-valeric acid (mol.%)	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	1.70 ± 0.72	1.16 ± 0.50	0.91 ± 0.57	0.47 ± 0.24

Inulin seems to have the potential to influence the gastrointestinal microbiota of suckling piglets through the diet of their mother

Two animal experiments

Wheat bran (insoluble fibers)
Vs. control

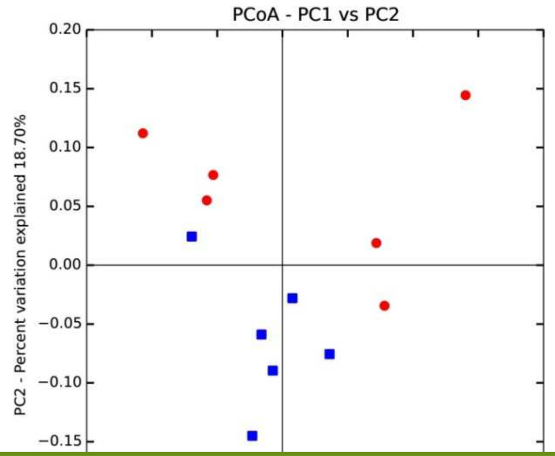
Pea starch (resistant starch)
Vs control

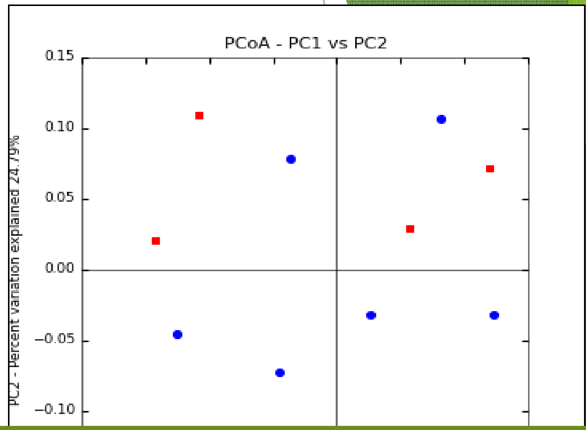
Gestation
Lactation

Gestation

Lactation

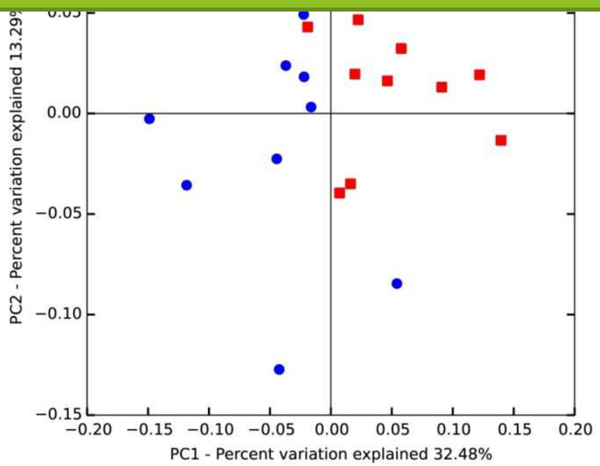
Wheat bran

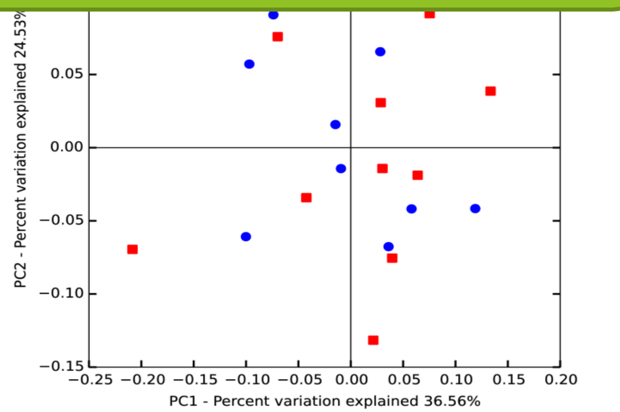




Clustering per treatment during gestation, but not during lactation

Pea starch





Gestation Wheat bran				
Genus	CON	WB	P-values	FDR
Bacteroidetes				
Parabacteroides	0.36	0.14	<0.001	0.02
Unclassified_Bacteroidales	6.13	2.25	<0.001	0.02
Bacteroides	0.22	0.04	<0.005	NS
CF231	1.22	0.57	0.01	NS
Unclassified_RF16	2.38	0.79	0.03	NS
Prevotella	15.5	19.0	NS	NS
Unclassified_ Erysipelotrichaceae OTU1	0.02	0.06	0.01	NS
Anaerovibrio	0.20	0.53	0.03	NS
Turicibacter	0.13	0.07	0.03	NS
Oscillospira	2.69	1.76	0.03	NS
Unclassified_Erysipelotrichaceae OTU2	0.08	0.03	0.06	NS
Unclassified_Mogibacteriaceae	0.75	0.44	0.07	NS
Proteobacteria				
Unclassified_Enterobacteriaceae	0.04	0.01	0.01	NS
Ruminobacter	0.02	0.03	NS	NS

13 genera differed in relative abundance between the CON and WB groups

Genus	Gestation Pea starch DS	RS	P	FDR
Actinobacteria				
Bifidobacterium	0.92	1.36	0.02	NS
Bacteroidetes				
Unclassified_RF16	1.53	0.80	0.01	NS
Firmicutes				
Unclassified_Ruminococcaceae	17.75	20.68	0.02	NS
Turicibacter	[illegible]	[illegible]	[illegible]	[illegible]
Sharpea	0.21	0.79	0.03	NS
[illegible]ae	0.12	0.20	0.02	NS
Spirochaetes				
Treponema	4.20	3.10	0.01	NS
Sphaerochaeta	1.05	0.50	<0.005	NS

Differences at the phylum level and the genus level during gestation

Dietary interventions on sows affect their fecal microbiota

WB exp, milk composition

Period	Treatment	Protein (%)	Fat (%)	Lactose (%)	IgA (mg/ml)	IgG (mg/ml)	IgM (mg/ml)
	CON	19.1	6.33	2.60	13.9	63.5	4.84
Colostrum	WB	19.0	6.45	2.62	13.4	68.5	4.22
	SEM	0.30	0.14	0.03	0.81	3.46	0.32
	CON	6.17	9.70	4.66	2.10	0.40	1.08
Milk W1[1]	WB	5.96	9.48	4.74	2.53	0.41	1.15
	SEM	0.09	0.51	0.05	0.16	0.05	0.11
	CON	6.03	9.84	4.81	2.43	0.30	1.10
Milk W2	WB	5.62	9.62	4.89	2.66	0.25	1.08
	SEM	0.10	0.28	0.02	0.17	0.02	0.07
	CON	6.22	9.72	4.82	3.41	0.20	0.97
Milk W3	WB	5.86	8.85	4.93	3.57	0.16	1.01
	SEM	0.08	0.38	0.03	0.23	0.02	0.08
	treatment	0.14	0.46	0.03	0.88	0.47	0.58
P-values	time	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	treatment*time	0.75	0.62	0.88	0.81	0.78	0.35

[1]W1= first week after farrowing, W2= second week, W3= third week

RS exp, milk composition

- Resistant starch:
  - Decreased protein concentration (all time points)
  - Increased lactose concentration in colostrum
  - Decreased lactose concentration on W3

Dietary interventions on sows affect milk macronutrient composition

WB exp, microbiota of piglets

	CON (N=7)	WB (N=7)	P-value	FDR
Actinobacteria	0.71	0.57	NS	NS
Collinsella	0.29	0.08	**0.04**	NS
Bacteroidetes	32.3	28.4	NS	NS
Butyricimonas	0.15	0.02	0.07	NS
Odoribacter	0.25	0.02	0.07	NS
Bacteroides	6.72	2.21	NS	NS
Unclassified_*Bacteroidales*	3.27	5.61	NS	NS
Prevotella	12.3	11.8	NS	NS
Euryarchaeota	0.01	0.02	**0.05**	NS
Methanobrevibacter	0.01	0.02	**0.05**	NS
Firmicutes	56.0	63.2	NS	NS
Unclassified_*Clostridiaceae*	1.57	2.82	**<0.001**	**0.04**
Unclassified_Lachnospiraceae OTU2	1.91	4.14	**0.04**	NS
Ruminococcus	1.74	0.85	0.07	NS
Phascolarctobacterium	2.35	3.68	0.07	NS
Roseburia	0.11	0.57	0.09	NS
Lactobacillus	14.8	13.1	NS	NS
Unclassified_*Clostridiales*	6.57	6.97	NS	NS
Unclassified_*Ruminococcaceae*	11.7	14.3	NS	NS

RS exp, microbiota of piglets

- The maternal diet did not affect colonic microbiota composition at weaning

Dietary interventions on sows has rather limited effects on piglet's microbiota

Treatment during the lactation period

Inulin

Inulin supplementation during the lactation period

12 litters with
6 piglets per
litter

4 litters-Control
(water)

4 litters-20% inulin
solution

4 litters-30% inulin
solution

After
weaning

d-28

Same diet
(no inulin)
for another
3 weeks

* Inulin was obtained by oral ingestion :
1st week: 2.5ml per day
2nd week: 5ml per day
3rd week: 7.5ml per day
4th week: 10ml per day

Temporary effects on microbiota

Effects on microbiota did not remain in the early post-weaning period

									M	*P*
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.410
Lactobacillus spp.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.540
Clostridium spp.	0.73	0.53	1.20	0.13	0.088	0.19	0.15	0.13	0.04	0.883
Escherichia spp.	1.22[b]	0.40[a]	1.24[b]	0.17	0.047	0.01	0.02	0.02	0.00	0.883
Enterobacteria spp.	1.25[ab]	0.40[a]	1.48[b]	0.17	0.014	0.04	0.05	0.02	0.02	0.730

Take home message

- Piglet’s microbiota is affected by:
  - Breed, origin
  - Environment
  - Sow’s microbiota
  - Sow’s milk composition
  - (Medical) treatments and stress
  - Diet
- Effects on gut homeostasis are observed
- Dietary interventions with fermentable feed ingredients affect microbiota, but sometimes to a limited extend