

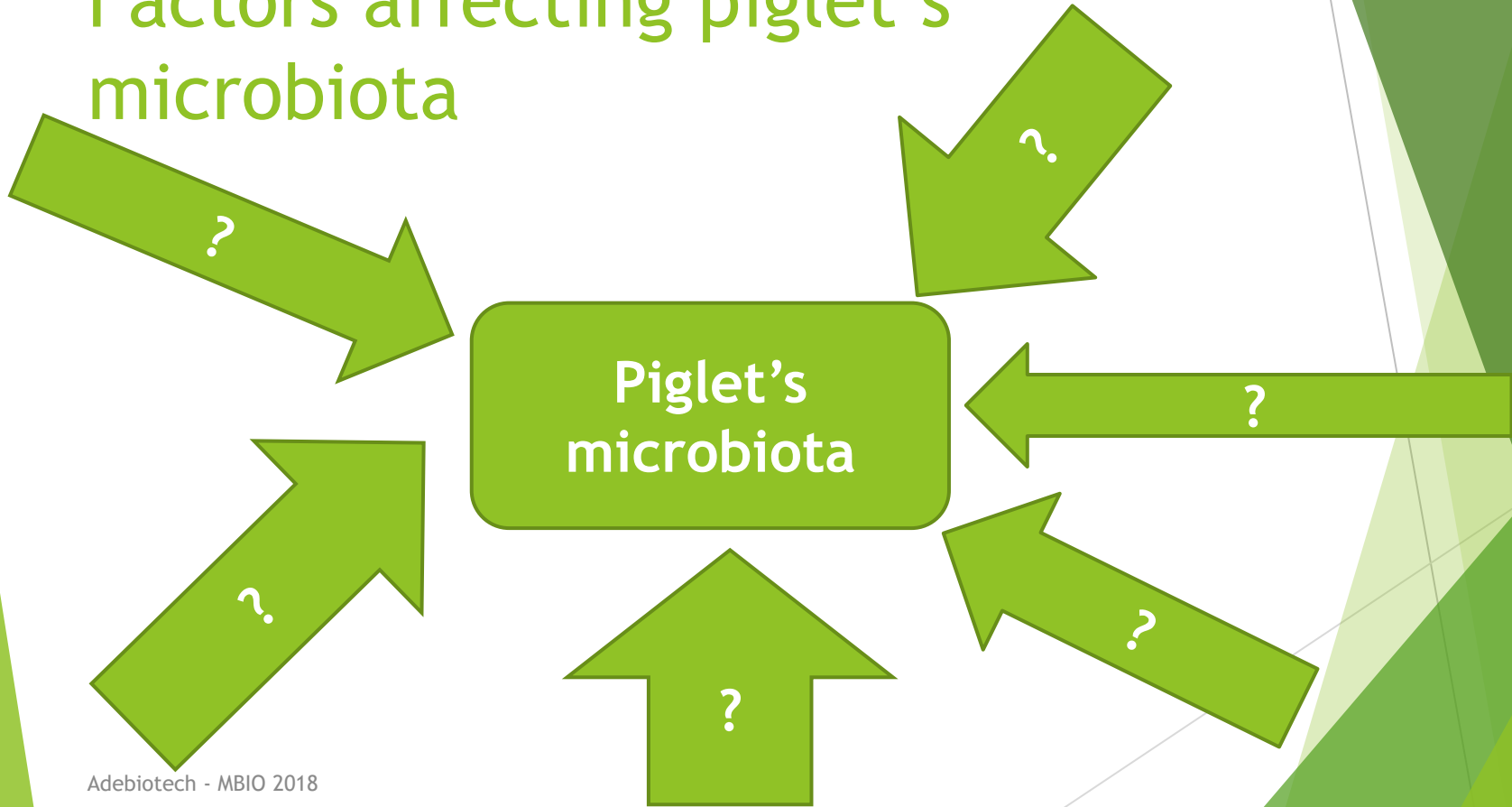


LIÈGE université
Gembloux
Agro-Bio Tech

Effects of prebiotics on the stability of the microbiota of the pig

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Factors affecting piglet's microbiota



Environment

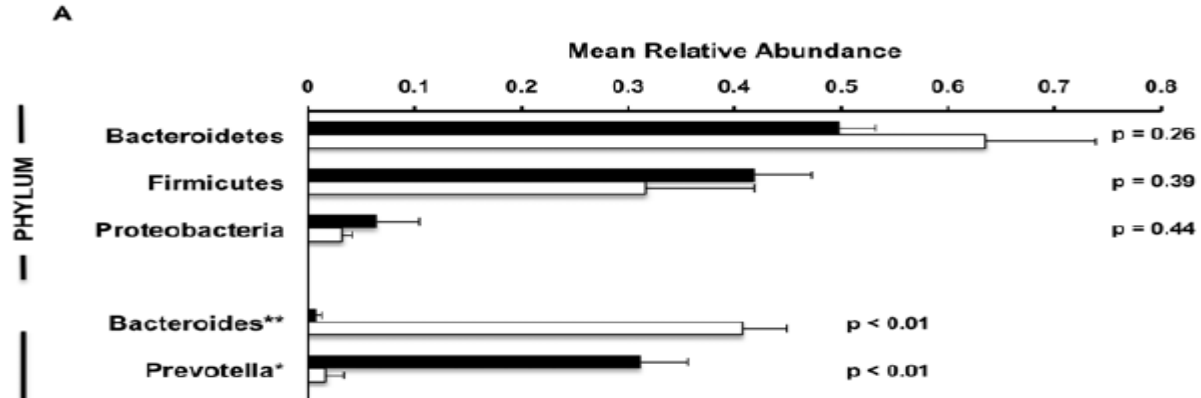
- ▶ Pigs separated from sow after birth and reared on milk replacer
- ▶ Non-siblings were co-housed in pairs
- ▶ Significant **correlation** of microbial communities

Gut colonization in piglets is greatly influenced by the immediate environment

Mother-fed vs formula MILK

D21

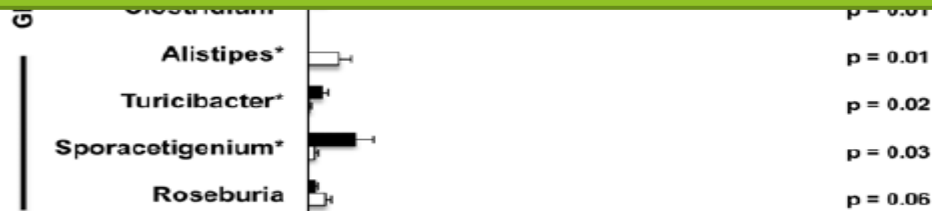
Cecal content



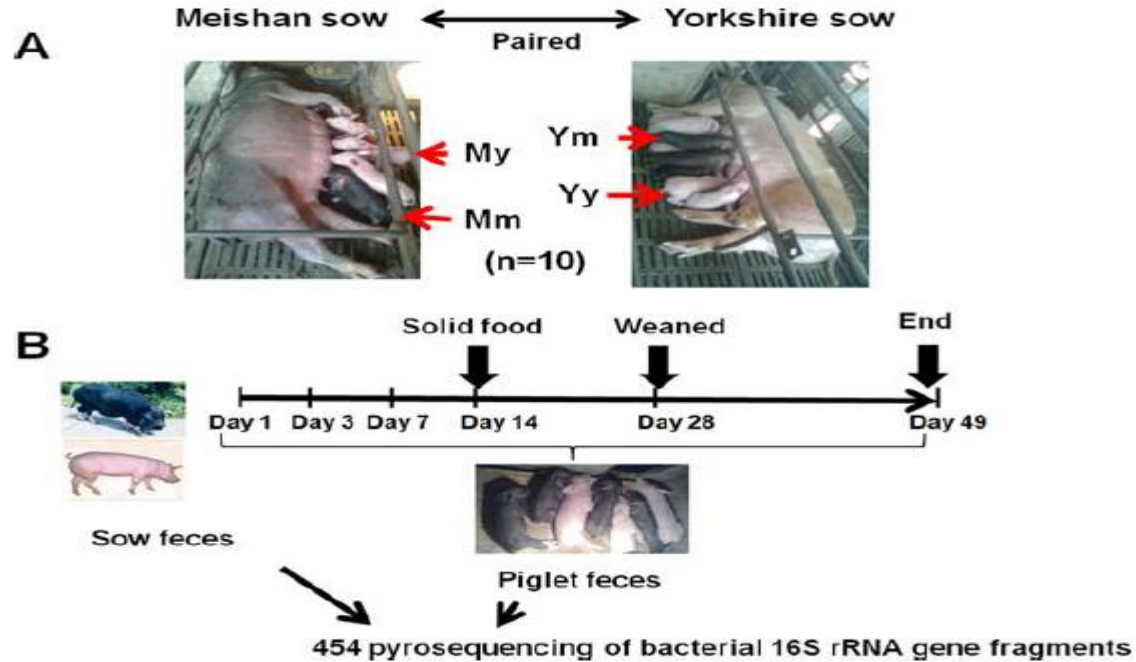
Black: mother fed

White: formula fed

Diet (milk) affects microbiota composition



Breed and nursing MOTHER



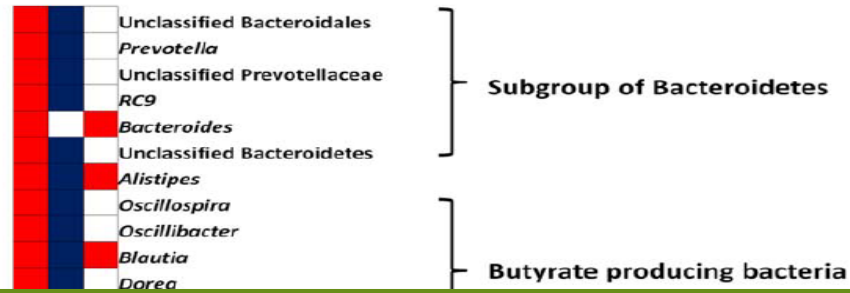
Effects of breed and nursing mother



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

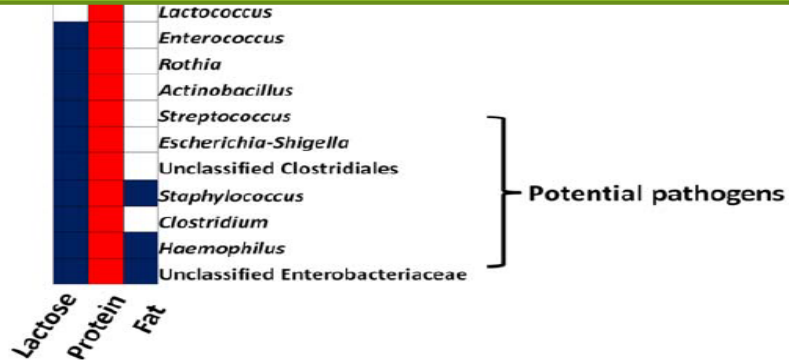


Impact of milk composition



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

Red: positive correlation
Blue: negative correlation



Antibiotic or stress-treatment on

d4
D8

Jejunal content

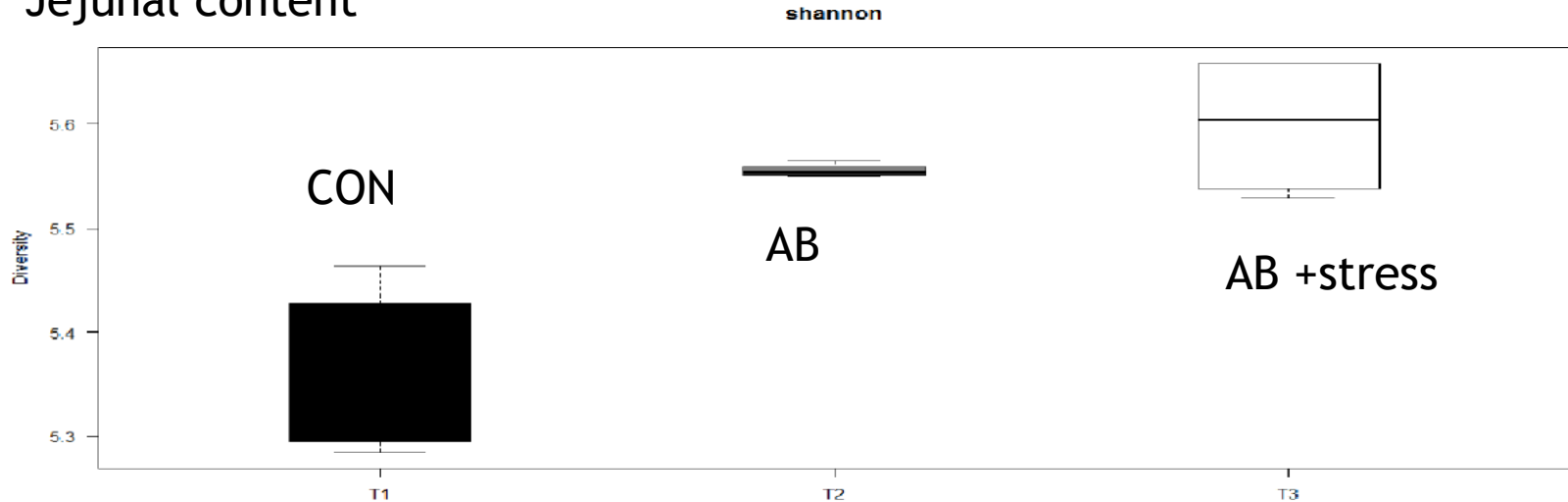


Figure 2. Diversity in microbiota in the three treatment groups. The Shannon index (y-axis) was calculated for all three treatments (T1, T2, and T3) (x-axis).

doi:10.1371/journal.pone.0100040.g002

Adebiotech - MBIO 2018

Schokker et al., 2014

Impact on microbiota composition

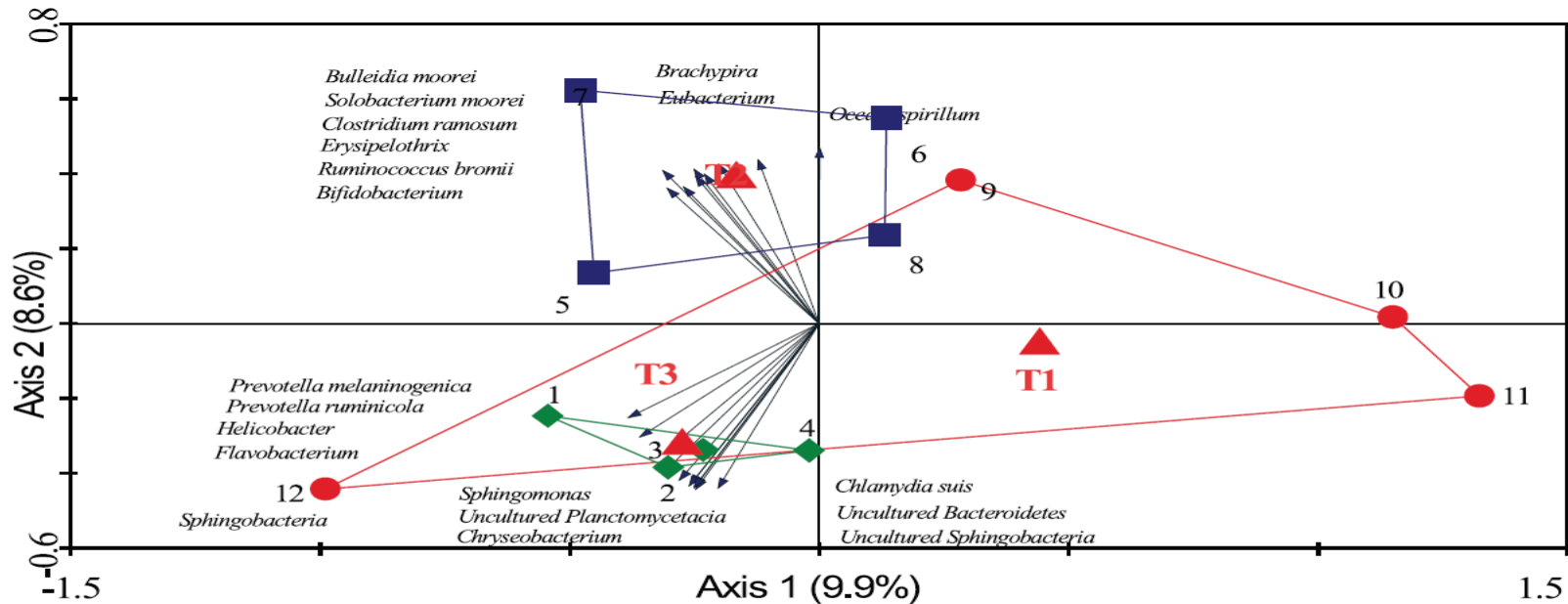
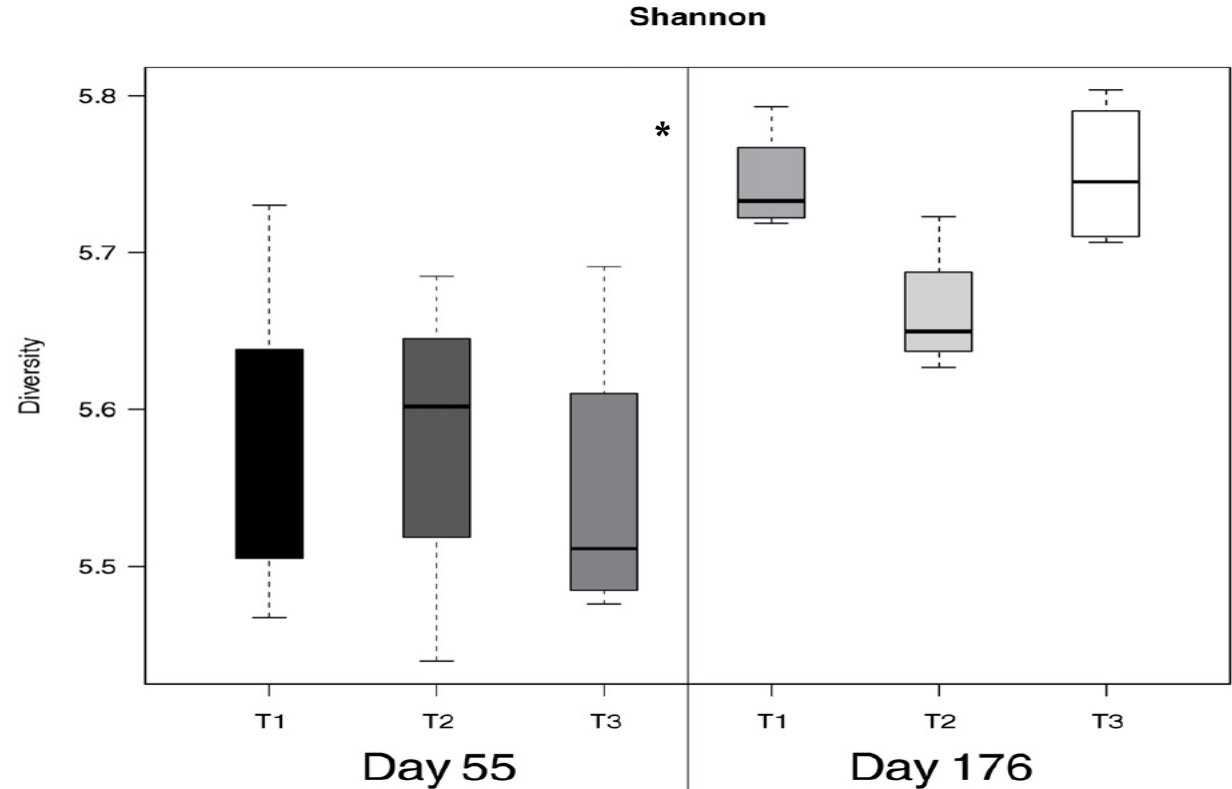


Figure 1. Triplot for RDA analysis of jejunal microbiota composition. Nominal environmental variables T1, T2 and T3 are represented by red triangles (▲). Samples are grouped by treatment: T1 (red; ○), T2 (blue; □) and T3 (green; ◇), each symbol represents a pool of four pigs, and numbers represent pool 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

Long-lasting effects on microbiota

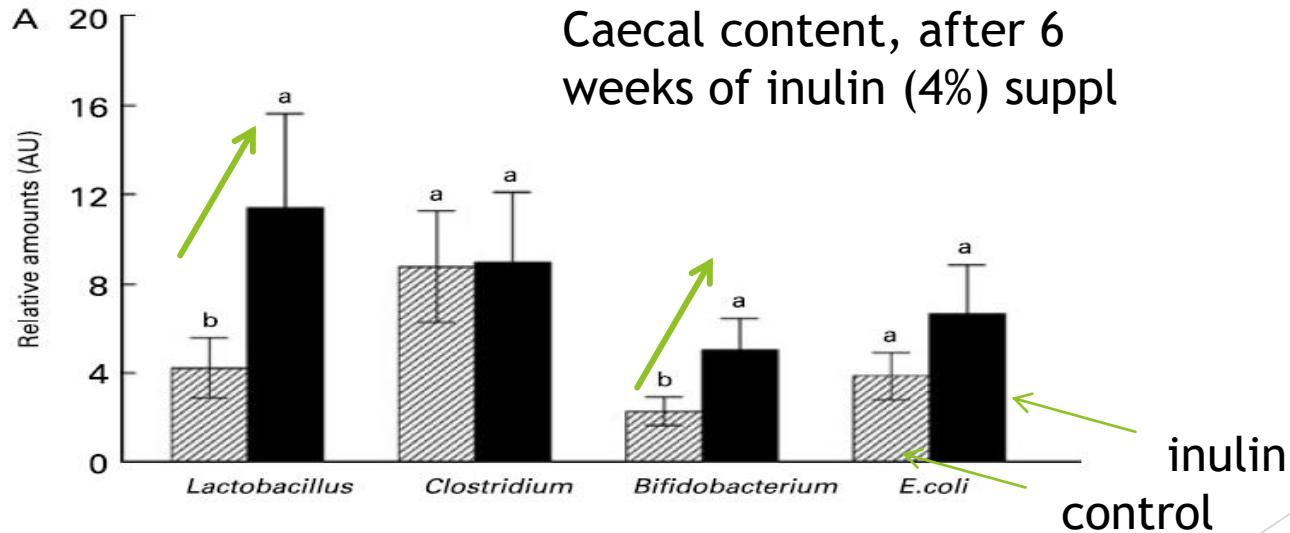
Jejunal content



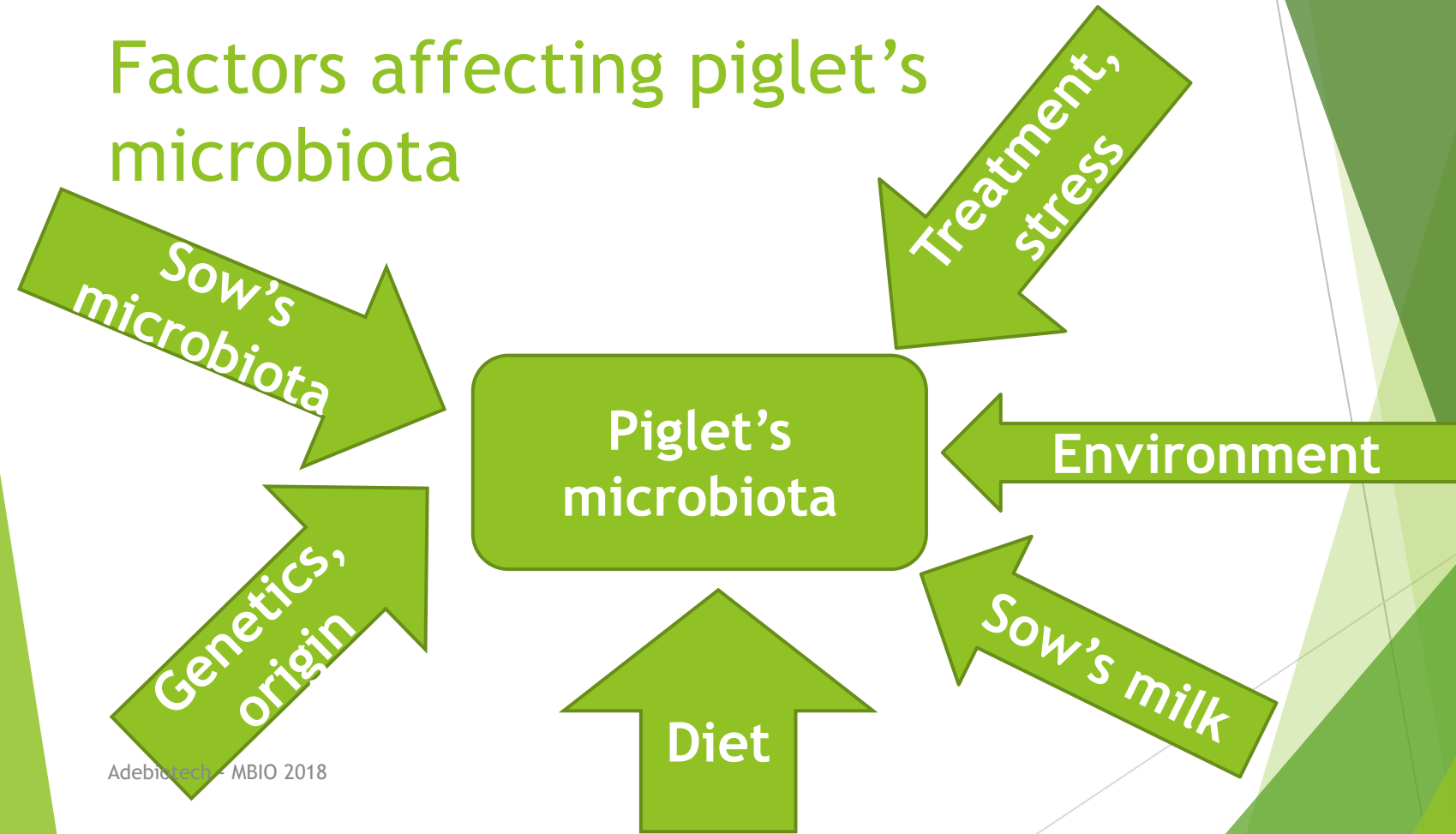
Adebiotech - MBIO 2018

Schokker et al., 2015

Inulin supplementation in weaning diet



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

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

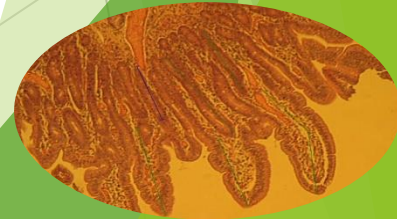
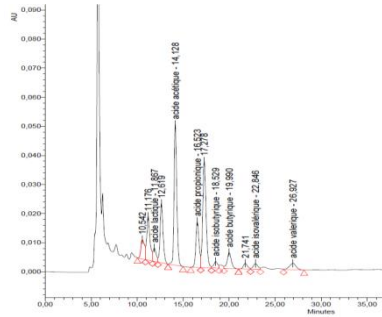
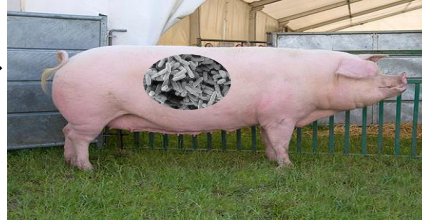
Effects on microbiota did not remain in the early post-weaning period

									M	P
<i>Lactobacillus spp.</i>	0.82	0.82	0.82	0.18	0.887	0.82	0.15	0.13	0.78	0.410
<i>Clostridium spp.</i>	0.73	0.53	1.20	0.13	0.088	0.19	0.15	0.13	0.04	0.883
<i>Escherichia spp.</i>	1.22 ^b	0.40 ^a	1.24 ^b	0.17	0.047	0.01	0.02	0.02	0.00	0.883
<i>Enterobacteria spp.</i>	1.25 ^{ab}	0.40 ^a	1.48 ^b	0.17	0.014	0.04	0.05	0.02	0.02	0.730

Effects by the maternal diet

Inulin
Wheat bran
Resistant starch

Hypotheses



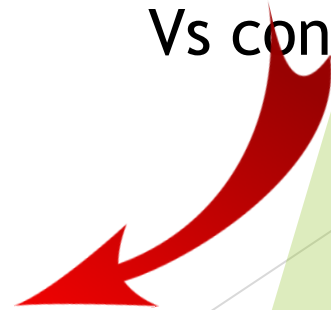
Two animal experiments



Wheat bran (insoluble
fibers)
Vs. control



Pea starch (resistant starch)
Vs control

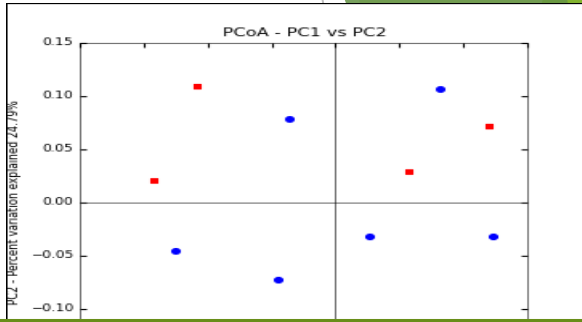
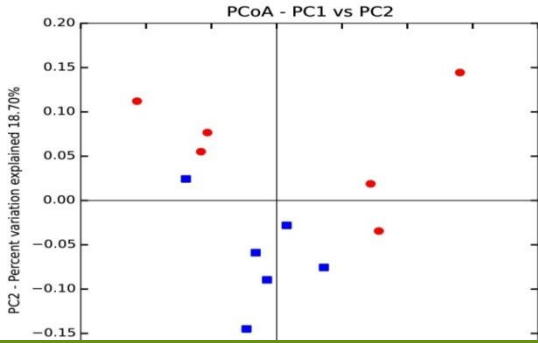


Gestation
Lactation

Gestation

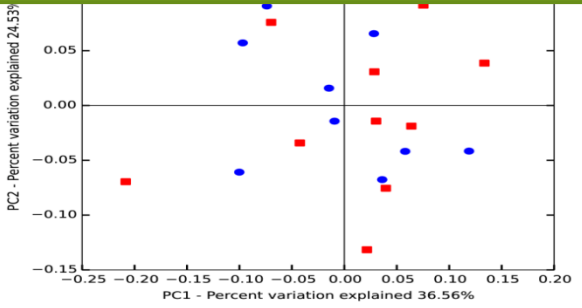
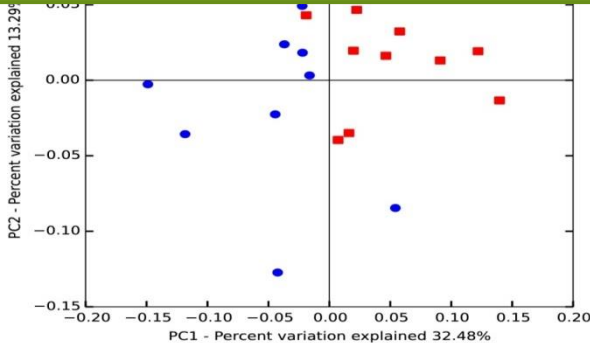
Lactation

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
Firmicutes				

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

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

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

Differences at the phylum level and the genus level during gestation

Sharpea	0.21	0.79	0.03	NS
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Dietary interventions on sows affect their fecal microbiota

Unclassified_Peptostreptococcaceae	0.12	0.20	0.02	NS
Spirochaetes				
Treponema	4.20	3.10	0.01	NS
Sphaerochaeta	1.05	0.50	<0.005	NS

WB exp, milk composition

Period	Treatment	Protein (%)	Fat (%)	Lactose (%)	IgA (mg/ml)	IgG (mg/ml)	IgM (mg/ml)
Colostrum	CON	19.1	6.33	2.60	13.9	63.5	4.84
	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
Milk W1 ¹	CON	6.17	9.70	4.66	2.10	0.40	1.08
	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
Milk W2	CON	6.03	9.84	4.81	2.43	0.30	1.10
	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
Milk W3	CON	6.22	9.72	4.82	3.41	0.20	0.97
	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
P-values	treatment	0.14	0.46	0.03	0.88	0.47	0.58
	time	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	treatment*time	0.75	0.62	0.88	0.81	0.78	0.35

¹W1= 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
<i>Butyricimonas</i>	0.15	0.02	0.07	NS
<i>Odoribacter</i>	0.25	0.02	0.07	NS
<i>Bacteroides</i>	6.72	2.21	NS	NS
Unclassified_Bacteroidales	3.27	5.61	NS	NS
<i>Prevotella</i>	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
<i>Ruminococcus</i>	1.74	0.85	0.07	NS
<i>Phascolarctobacterium</i>	2.35	3.68	0.07	NS
<i>Roseburia</i>	0.11	0.57	0.09	NS
<i>Lactobacillus</i>	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 have rather limited effects on piglet's microbiota

Take home message

- ▶ Piglet's microbiota is affected by:
 - ▶ Breed, origin
 - ▶ Environment
 - ▶ Sow's microbiota
 - ▶ Sow's milk composition
 - ▶ (Medical) treatments and stress
 - ▶ Diet
- ▶ Early in life, dietary interventions with fermentable feed ingredients affect microbiota, but sometimes to a limited extent, and fading out when treatments stopped

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Thank you!