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## Impact of weaning age on the gut microbiota composition in piglets.

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Weaning is one of the most important period of pig life

- ➢ 3<sup>rd</sup> and 4<sup>th</sup> week of age
- > switch from highly digestible liquid milk to a less-digestible more-complex solid feed
- move from maternity building to a post-weaning unit
- > social change owing to the separation from the mother and by mixing piglets from different litters







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Piglets during the weaning are susceptible to diarrhea:

- ✓ Dysbiosis
- ✓ Colonization of enteric pathogens







The gastrointestinal microbiota is dynamic and subject to changes based on environment, age, exposure to microbes and diet.

The gut microbiota is mostly stressed at weaning.

The main debated parameter is the age at weaning and few studies have been performed about how earlylife establishment of the swine gut microbiota may contribute during the weaning period to the individual's robustness.





- ✓ To characterize the gut microbiota composition at different weaned ages, ranging from ultra-early weaning (14 days) to organic-like weaning (42 days), in antibioticfree piglets
- ✓ To evaluate the effect of weaning age on the later composition of the gut microbiota (day 60)





N. piglets	Weanead at	Birth (d0)	d14	d21	d28	d35	d42	d49	d60
12	14 days		Faeces (12 pigs)	Faeces (12 pigs)					Faeces (6 pigs)
12	21 days		<b></b>	Faeces (12 pigs)	Faeces (12 pigs)				Faeces (6 pigs)
12	28 days				Faeces (12 pigs)	Faeces (12 pigs)		→	Faeces (6 pigs)
12	42 days					<b>→</b>	Faeces (12 pigs)	Faeces (12 pigs)	Faeces (6 pigs)

 $\checkmark\,$  Animals were recorded for growth and diarrhea



## > MATERIALS AND METHODS

- ✓ DNA extraction
- ✓ 16S sequencing using the Illumina system
- ✓ Bioinformatics data analyses using QIIME (v1.9.1)
  - Samples < 10,000 post-quality reads were removed from the analysis</li>
- ✓ Biostatistical analyses using R software
  - PhyloSeq
  - Vegan
  - MetagenomeSeq



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## > RESULTS

### GROWTH

Impact of weaning on growth rate decreased with weaning age, and for W42 the ADG was stable and increased

### DIARRHEA

Weaning at younger ages increased diarrhea, which was not observed in W42 group









Alphadiv. Post Weaning



# RESULTS – Diversity analysis Alpha diversity





# RESULTS – Diversity analysis Alpha diversity

The group weaned at 42 days old has a higher alpha diversity during the pre-weaning, postweaning and until the day 60

These animals having a richer microbiota can be more resistant to the enteric diseases during the weaning period and for all their lifelong (Dou *et al.*, 2017)



#### Betadiv. Pre Weaning

Betadiv. Post Weaning

Betadiv. Day 60

w42\_d60

v28\_d60



# RESULTS – Diversity analysis Beta diversity

The lower beta diversity in the w42 group means that the population of the w42 is more homogeneous than the other weaned groups that are heterogeneous until day 60.

The adult microbiota is known to be more stable than younger microbiota (Edwards *et al.*, 2017)



#### Bray-Curtis distance Weaning\_d14

Bray-Curtis distance Weaning\_d21



### RESULTS – NMDS analysis

Each weaned group revealed significant differences between the samples point using the NMDS analysis

The gut microbiota changes drastically before and after weaning, as already demonstrated in previous studies (Mach *et al.*, 2015;Dou *et al.*, 2017)





NMDS (Bray-Curtis distance) OTU counts by Day (Day\_d60)



### **RESULTS – NMDS analysis**

Pre and post-weaned groups revealed significant differences between days but none statistical significance was revealed in the day 60 group.



## **RESULTS – Differential abundant analysis**



Post-Weaning:

Actinobacillus spp., Anaerotruncus spp., Dialister spp., Mucispirillum spp., Prevotella spp., Blautia spp., Faecalibacterium prausnitzii



## RESULTS – Differential abundant analysis





Front Microbiol. 2017 Jun 30;8:1226. doi: 10.3389/fmicb.2017.01226. eCollection 2017.

# Functional Characterization of Novel Faecalibacterium prausnitzii Strains Isolated from Healthy Volunteers: A Step Forward in the Use of F. prausnitzii as a Next-Generation Probiotic.

Martín R<sup>1</sup>, Miquel S<sup>1,2</sup>, Benevides L<sup>1,3</sup>, Bridonneau C<sup>1</sup>, Robert V<sup>1</sup>, Hudault S<sup>1</sup>, Chain E<sup>1</sup>, Berteau O<sup>1</sup>, Azevedo V<sup>3</sup>, Chatel JM<sup>1</sup>, Sokol H<sup>1,4,5</sup>, Bermúdez-Humarán LG<sup>1</sup>, Thomas M<sup>1</sup>, Langella P<sup>1</sup>.



### *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients

Harry Sokol<sup>\*†</sup>, Bénédicte Pigneur<sup>†‡</sup>, Laurie Watterlot<sup>\*</sup>, Omar Lakhdari<sup>\*</sup>, Luis G. Bermúdez-Humarán<sup>\*</sup>, Jean-Jacques Gratadoux<sup>\*</sup>, Sébastien Blugeon<sup>\*</sup>, Chantal Bridonneau<sup>\*</sup>, Jean-Pierre Furet<sup>\*</sup>, Gérard Corthier<sup>\*</sup>, Corinne Grangette<sup>§</sup>, Nadia Vasquez<sup>¶</sup>, Philippe Pochart<sup>¶</sup>, Germain Trugnan<sup>‡</sup>, Ginette Thomas<sup>‡</sup>, Hervé M. Blottière<sup>\*</sup>, Joël Doré<sup>\*</sup>, Philippe Marteau<sup>|</sup>, Philippe Seksik<sup>±\*\*+†</sup>, and Philippe Langella<sup>\*,\*+††</sup>

Gastroenterol Res Pract. 2014;2014:872725. doi: 10.1155/2014/872725. Epub 2014 Mar 27.

Association between Faecalibacterium prausnitzii Reduction and Inflammatory Bowel Disease: A Meta-Analysis and Systematic Review of the Literature.

Cao Y<sup>1</sup>, Shen J<sup>1</sup>, Ran ZH<sup>1</sup>.

Appl Environ Microbiol. 2015 Nov;81(21):7582-92. doi: 10.1128/AEM.02006-15. Epub 2015 Aug 21.

Mucosa-associated Faecalibacterium prausnitzii phylotype richness is reduced in patients with inflammatory bowel disease.

Lopez-Siles M<sup>1</sup>, Martinez-Medina M<sup>1</sup>, Abellà C<sup>1</sup>, Busquets D<sup>2</sup>, Sabat-Mir M<sup>3</sup>, Duncan SH<sup>4</sup>, Aldeguer X<sup>2</sup>, Flint HJ<sup>4</sup>, Garcia-Gil LJ<sup>5</sup>.





We show that late weaning leads to:

- > higher diversity of potentially beneficial microbes prior to the crucial challenge of weaning
- might provide a competitive advantage to piglets

**FUTURE STUDIES:** What will be the impact of a late weaning in the long term life of pigs?





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# THANKS FOR THE ATTENTION!!!!

