

# Effect of *Saccharomyces cerevisiae boulardii* supplementation on gut microbiota in post-weaning piglets in a context of antibiotics and ZnO removal

C. Achard<sup>1</sup>, F. Bravo de Laguna<sup>1</sup>, M. Castex<sup>1</sup>, S. Combes<sup>2</sup>, A. Agazzi<sup>3</sup>, V. Bontempo<sup>3</sup>, M. Comi<sup>3</sup> and V. Perricone<sup>3</sup>, G. Savoini<sup>3</sup>

<sup>1</sup>Lallemand Animal Nutrition, France

<sup>2</sup>GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet-Tolosan, France

<sup>3</sup>University of Milan, Department of Health, Animal Science and Food Safety, Milano, Italy

## Background and objectives

In a context of reduction of antibiotics and zinc oxide (ZnO), alternatives are needed to maintain animal health and growth performance. In a study conducted in post-weaning piglets, we have demonstrated positive effects of a supplementation with *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 (SB,  $2 \times 10^9$  CFU/kg). Despite a removal of antibiotics and ZnO after 11 days post-weaning, SB supplemented piglets displayed the same average daily gain (ADG) and feed conversion rate (FCR). Thus SB could help to face post weaning challenges (Agazzi, 2017). The microorganisms that inhabit the gastrointestinal tract contribute to host health and performance. The objective of this study was thus to assess whether the gut microbiota could be beneficially affected by SB supplementation and suppression of medication.

## Material and methods

216 fecal samples were collected from weaned piglets during the trial performed as follows: piglets were weaned at 21 day of age and followed a 3-phase feeding program. Basal diet was supplemented with antibiotics and ZnO in the first two phases (D0-D11 and D12-D33) and only with antibiotics in the third phase (D34-D50). Piglets were allocated to three groups: basal diet (Ctl), basal diet supplemented with  $2 \times 10^9$  CFU/kg SB (T1), diet without medication after D11 and with SB (T2). Microbial DNA was extracted from feces and high-throughput sequencing of the V4 and V5 regions of the 16S rRNA gene was performed on MiSeq sequencer. Sequences were processed using FROGS pipeline: 1) Clustering in Operational Taxonomic Units (OTUs) using SWARM, 2) chimera detection using UCHIME and 3) taxonomic annotation using the SILVA128 database. Statistical analyses were carried out using R software. Mixed linear model accounting for the fixed effect of the sampling date, the treatment, their interaction and the random effect of the animal was applied to analyze microbiota composition. ANOVA and Tukey's multiple comparisons of means were performed. A Kruskal-Wallis Rank Sum Test followed by Pairwise Test for Multiple Comparisons of Mean Rank Sums were used to analyze microbiota data that did not fulfilled ANOVA prerequisites. P values were adjusted with Benjamini-Hochberg procedure. Vegan package was used to generate non Metric Dimensional Scaling (nMDS) ordination on OTUs Bray-Curtis dissimilarity matrix and perform multivariate analysis of variance. Sparse Partial Least Square Discriminant Analysis, was applied to identify the OTUs that contribute the most to discriminate the samples according to the treatment.

## Results

Alpha-diversity was significantly increased at D34 when compared with D10. No further increase was evidenced at D50. Alpha-diversity was not affected by treatment. The microbiota composition was significantly changed over time. Unsupervised multivariate analysis highlighted significant differences between Ctl and T1 groups at D10, between Ctl and T2 at D34 and between Ctl and T1 and T2 at D50. SB supplementation was associated with increased *Fibrobacteres* abundance (in T2 group) and with increased *Coriobacteriaceae* family at D10 which can respectively exhibit fibrolytic and mucin degradation activities. A discriminant analysis allowed us to point out 190 OTUs that contribute to the discrimination of the treatment groups at D50. Interestingly, some beneficial bacteria (*Roseburia*, *Subdoligranulum*, *Anaerotruncus*, *Faecalibacterium*) were less abundant in Ctl group.

## Conclusion and discussion

Changes of diet and medication associated with ZnO removal in the third phase was associated with important changes in gut microbiota composition. Beneficial effects on performance of SB supplementation with and without medication were associated with beneficial changes in microbiota composition. Medication and SB supplementation differently shape gut microbiota suggesting function redundancy of the microbiota or different mode of action involved in improvement of growth performances.

## References

Agazzi et al., EAAP 2017, Tallinn, Estonia.