Effect of live yeast *Saccharomyces cerevisiae* supplementation on the performance and cecum microbial profile of suckling piglets

Tadele G. Kiros¹*, Diana Luise²*, Hooman Derakhshani³, Renee Petri¹, Paolo Trevisi²*, Romain D’Inca⁴, Eric Auclair⁴, Andrew G. van Kessel¹*

1 University of Saskatchewan, Department of Animal and Poultry Science, Saskatoon, Saskatchewan, Canada, 2 Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, 3 Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada, 4 Phileo-Lesaffre Animal Care, Marcq-en-Baroeul, France

*These authors contributed equally to this work.

* andrew.vankessel@usask.ca (AK); paolo.trevisi@unibo.it (PT)

Abstract

One mechanism through which *S. cerevisiae* may improve the performance of pigs is by altering the composition of the gut microbiota, a response that may be enhanced by early postnatal supplementation of probiotics. To test this hypothesis, newborn piglets (16 piglets/group) were treated with either *S. cerevisiae* yeast (5 x 10⁹ cfu/pig: Low) or (2.5 x 10¹⁰ cfu/piglet: High) or equivalent volume of sterile water (Control) by oral gavage every other day starting from day 1 of age until weaning (28 ± 1 days of age). Piglet body weight was recorded on days 1, 3, 7, 10, 17, 24 and 28 and average daily gain (ADG) calculated for the total period. At weaning, piglets were euthanized to collect cecum content for microbial profiling by sequencing of the 16S rRNA gene. ADG was higher in both Low and High yeast groups than in Control group (*P* < 0.05). Alpha diversity analyses indicated a more diverse microbiota in the Control group compared with Low yeast group; the High yeast being intermediate (*P* < 0.01). Similarly, Beta diversity analyses indicated differences among treatments (*P* = 0.03), mainly between Low yeast and Control groups (*P* = 0.02). The sparse Partial Least Squares Discriminant Analysis (sPLS-DA) indicated that Control group was discriminated by a higher abundance of *Veillonella*, *Dorea*, *Oscillospira* and *Clostridium*; Low yeast treated pigs by higher *Blautia*, *Collinsella* and *Eubacterium*; and High yeast treated pigs by higher *Eubacterium*, *Anaerostipes*, *Parabacteroides*, *Mogibacterium* and *Phascolarctobacterium*. Partial Least Squares (PLS) analysis showed that piglet ADG was positively correlated with genus *Prevotella* in High yeast group. Yeast supplementation significantly affected microbial diversity in cecal contents of suckling piglets associated with an improvement of short chain fatty acid producing bacteria in a dose-dependent manner. In conclusion, yeast treatment improved piglet performance and shaped the piglet cecum microbiota composition in a dose dependent way.
the manuscript. The specific roles of these authors are articulated in the ‘author contributions’ section.

Competing interests: The authors have read the journal’s policy and have the following competing interests: EA is an employee of Phileo Lesaffre Animal Care. RD was an employee of Phileo Lesaffre Animal Care at the time the study was conducted. TK was an employee of the University of Saskatchewan during the time the study was conducted. At the time of submission, TK was also employed by Phileo Lesaffre Animal Care. Phileo Lesaffre Animal Care provided financial support which was partly used to purchase materials for the study in the form of animal and laboratory supplies. This does not alter our adherence to PLOS ONE policies on sharing data and materials. There are no patents, products in development or marketed products to declare.

Abbreviations: ADG, Average daily gain; ETEC, Enterotoxigenic Escherichia coli; NMDS, Non-Metric Multidimensional Scaling Plots; OTUs, Operational Taxonomic Units; PLS, Partial Least Squares; SCFA, Short chain fatty acids; sPLS-DA, Sparse Partial Least Squares Discriminant Analysis.

Introduction

The use of Saccharomyces cerevisiae (S. cerevisiae) yeast as a feed additive for farm animals has increased enormously in the last decade, especially after the ban of antibiotic growth promoters in Europe. In ruminants, S. cerevisiae is reported to increase feed efficiency [1] and improve milk production, as well as milk quality [2,3]. Data from monogastric animals have been contradictory; some reporting no effect on feed intake or body weight gain of sows [4] or weanling pigs [5], while others have reported improved milk quality in sows [6] and increased daily feed intake and body weight gain in weanling pigs [7,8,9] as well as the increasing resistance against Enterotoxigenic Escherichia coli (ETEC) F4 or Salmonella infection in piglets [10,11,12]. Similarly, daily supplementation of broiler chicken with live S. cerevisiae increased feed intake, body weight gain, feed conversion ratio, and improved carcass quality [13,14]. Despite the contradictory results coming from monogastric animal studies, the use of live yeast as a feed additive in the livestock industry is on the rise due to the general understanding that probiotics, including live yeast supplementation, improve the health and performance of farm animals [4,5]. However, the mode of action by which live yeast supplementation improves the overall health and performance of farm animals is not yet fully elucidated. Since the pig gut microbiota is considered to be unstable during the first 3 to 4 weeks of age [15], early supplementation of suckling piglets with a yeast probiotic may improve the stability of the gut microbiota before weaning and improve gut health and performance in newborn piglets. The objective of this study is, therefore, to evaluate the effect of S. cerevisiae (Actisaf CNCM I-4407) supplementation on the performance and composition of the cecum microbiota of piglets before weaning.

Material and methods

Ethics statement

The study was conducted, and animals handled according to the regulations and guidelines provided by the Canadian Council on Animal Care for humane animal use and approved by the University of Saskatchewan Animal Research Ethics Board under animal use protocol number AUP-20110132.

Experimental design

Fig 1 summarizes the experimental design. Eight sows were randomly selected among the newly farrowed sows at the Prairie Swine Center (University of Saskatchewan, Canada) and assigned to either Control or Yeast group, 4 sows per group balanced for parity. Control and Yeast group sows and their newborn piglets were housed in the same farrowing room on opposite side of the room to avoid cross contamination between Yeast and Control groups. Eight healthy looking piglets were chosen from each sow in the Yeast group and ear tagged for individual identification. Four piglets from each sow in the Yeast group (n = 16) received the regular dose (5 x 10⁸ cfu/piglet in a total volume of 3 mL; Low) of S. cerevisiae yeast (Actisaf, CNCMI-4407; Phileo-LeSaffre Animal Care, Marcq-en-Baroeul, France) recommended by the manufacturing company, while the remaining 4 piglets (n = 16) received a higher dose of yeast (2.5 x 10⁹ cfu/piglet in a total volume of 6 mL; High). Yeast solutions were prepared by mixing yeast powder in sterile water and administered to piglets by oral gavage using a 10 mL syringe. Four piglets from each of the Control group sows (n = 16) were also ear tagged and received equal volume of sterile water by gavage (Control). To the extent possible, piglets in each treatment group were balanced for weight and sex. Yeast dietary supplementation and the Control treatment were administrated every other day starting from day (d) 1 of age until weaning.
Control and yeast treated piglets were deliberately selected from different litters to avoid cross-contamination of Control piglets by yeast shedding from yeast supplemented piglets if they were to be kept with the same sow in the same pen throughout the study period.

During the lactation period, sows had free access to feed and water. Diet was formulated to meet or exceed the National Research Council [16] nutrient requirements for lactating sows. However, lactating sows did not get any yeast supplementation. Piglets had free access to water and no creep-feed was given during pre-weaning. The farrowing room temperature was maintained at 23 °C and heat lamps were used the first week after birth. After one week of age, the heat lamps were used only at night.

Sample collection

Piglet body weight (BW) was recorded on days 1, 3, 7, of age and then every week until weaning (day 28 ± 1). Control and yeast treated piglets were deliberately selected from different litters to avoid cross-contamination of Control piglets by yeast shedding from yeast supplemented piglets if they were to be kept with the same sow in the same pen throughout the study period.

During the lactation period, sows had free access to feed and water. Diet was formulated to meet or exceed the National Research Council [16] nutrient requirements for lactating sows. However, lactating sows did not get any yeast supplementation. Piglets had free access to water and no creep-feed was given during pre-weaning. The farrowing room temperature was maintained at 23 °C and heat lamps were used the first week after birth. After one week of age, the heat lamps were used only at night.

Effect of Saccharomyces cerevisiae supplementation on performance and cecum microbiota of suckling piglets

Fig 1. Experimental design. Eight sows were randomly selected among the newly farrowed sows at the Prairie Swine Center (University of Saskatchewan, Canada) and assigned to either Control or Yeast group, 4 sows per group balanced for parity. Eight healthy looking piglets (balanced for sex and body weight) were chosen from each sow in the Yeast group (n = 32) and assigned either to Low dose yeast group (n = 16; 4 piglets per sow) or to High dose yeast group (n = 16; 4 piglets per sow). Four healthy looking piglets (balanced for sex and body weight) were chosen from each sow in the Control group and ear tagged for individual identification (n = 16; 4 piglets per sow). Low dose piglets received the regular dose (5 x 10^9 cfu/piglet; Low) of S. cerevisiae yeast (Actisaf, CNCMI-4407; Phileo-LeSaffre Animal Care, Marcy-en-Baroeul, France) recommended by the manufacturing company, while High dose yeast piglets received a higher dose of yeast (2.5 x 10^10 cfu/piglet; High). Yeast solutions were prepared by mixing yeast powder in sterile water and administered to piglets by oral gavage using a 10 mL syringe. Piglets were individually weighted at days 1,3,7,10,17, 24,28 of age. On weaning day, piglets were euthanized to collect the cecum content to analyses the microbial profile.
and piglets’ ADG is included in S1 Table. At weaning, piglets were humanely killed by captive bolt stunning and pitching. From each pig (16 piglets/group) caecal content was collected and immediately stored at -20°C until analysis.

**Bacterial DNA extraction and purification**

Total bacterial DNA was extracted from 350 mg cecal contents by bead-beating method using the 25:24:1 phenol/chloroform/isoamyl alcohol extraction technique as previously described [17]. Extracted DNA was re-suspended in 500 μL nucleic acid free water and purified using the bead based ChargeSwitch gDNA purification kit (Invitrogen) according to the manufacturer’s protocol. The concentration of the DNA eluted in 200 μL of elution buffer was measured and purity determined by absorbance at 260 and 280 nm using the NanoDrop; ND-1000 Spectrophotometer. DNA samples were subjected to a deep sequence analysis (10,000 reads per sample) at the Molecular Research (MR DNA) laboratory in Shallow water, TX, USA.

**454 pyrosequencing**

Amplicon pyrosequencing (bTEFAP) originally described [18] was utilized for deep sequence analysis of intestinal content samples. Briefly, the 16S universal Eubacterial primers 27F (AGRGTTTGATCMTGGCTCAG) and 519R (GTNTTACNGCCGCGTGC) which amplify a sequence spanning the V1-V3 hypervariable regions were used together with short chain unique DNA sequence tags (barcodes) [19]. Around 100 ng (1μL) pure DNA was used per 50μL of PCR reaction. A single-step 30 cycles PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds; 53°C for 40 seconds and 72°C for 1 minute. The final elongation step was for 5 minutes at 72°C. Following PCR, all amplicon products from different samples were pooled in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced utilizing Roche 454 FLX titanium instrument and reagents (Roche, Nutley, New Jersey) following the manufacturer’s instructions.

**Sequence processing**

Sequence data derived from the sequencing process were subjected to a stringent quality control screening processes. Briefly, sequences were depleted of barcodes and primers, and then short sequences < 150bp, sequences with ambiguous base calls, and sequences with homopolymer run exceeding 6 bp were removed. The remaining reads were further screened and those with more than 3 consecutive bases with quality scores below 25 were truncated. Sequences were assigned to Operational Taxonomic Units (OTUs) using subsampled open-reference; average read length of OTUs was 436 nucleotides. OTU- picking was carried out using UCLUST with 97% sequence similarity on QIIME (v1.9.1). Singleton OTUs were removed and taxonomies were assigned to the representative sequence of each OTU using UCLUST and aligned with the latest Greengenes Core reference database (version 13.8) [20] using PyNAST algorithms [21]. Data were chimera checked using the Blast fragments approach [22] in QIIME Phylogenetic tree was built with FastTree 2.1.3 [23] for further comparison between microbial communities.

**Bioinformatics and statistical analysis**

Body weight was analyzed using an ANOVA mixed model in which age and treatment were included as fixed effects, while sow was included as random effect, and piglets as repeated measurement (by including random intercept terms) to account for multiple observations within
the same sow or within the same piglets. ADG was analyzed using an ANOVA mixed model in treatment was included as fixed effects, while sow was included as random effect, and piglets as repeated measurement. Tukey’s honest significance test was carried out at a 95% confidence level to test multiple comparison among group. Effects were considered significant when $P < 0.05$. Statistical analysis was performed with R statistical software (v.3.3.0) [24] using the "lme4" and "emmeans" packages.

The singletons and OTUs with relative abundance across all samples below 0.005% were removed as recommended by Bokulich [25]. Four samples that reported low sequencing yield (less than 4000 reads after quality check) were excluded from further analysis (three samples from CON group and one sample from Low yeast group). Number of sequences per sample ranged from 3982 to 18,200 and sequences were classified into 744 representatives OTUs. Bio-statistics on OTUs were performed using phyloseq [26], Vegan [27] and mixOmix [28] packages in R software (v.3.3.0) [24].

The richness (observed OTUs) and alpha diversity indices (Shannon and Chao indices (Chao, 1984 [29])) were calculated on raw data and comparison between treatments were tested using a mixed model including treatment as fixed factor and sow as random factor. Bray-Curtis Dissimilarity was calculated and used to compare the treatment differences in beta diversity using a permutational MANOVA (Adonis and pairwise.adonis procedure) including treatment and sow as factors. Non-Metric Multidimensional Scaling Plots (NMDS) based off Bray-Curtis Dissimilarity distances were visualized in R software [24] using ggplot2. Beta diversity ordination (Bray Curtis distance matrix) was calculated after rarefaction correction.

Taxonomic differences among groups were tested using Wilcoxon signed-rank test on OTUs aggregated data at Genus level. $P$-values were corrected for FDR correction. $P$-value $< 0.05$ was considered as significant and $P$-value $< 0.1$ was considered a trend of significance. Furthermore, to further investigate the differences in cecum microbiota composition between groups, the sparse Partial Least Squares Discriminant Analysis (sPLS-DA) was performed [30, 31]. It performs a variable (OTUs aggregated at Genus level) selection with Lasso penalization method. The optimal number of components were selected based on the averaged balanced classification error rate with centroids distance over 50 repeats of a 5-fold cross-validation of a sPLS-DA model with 3 components (using “perf” function). The optimal number of selected variables for each component was then chosen based on the lowest average balanced classification error rate with centroids after tuning of the sPLS-DA model (function tune.spldsda) using the selected number of components and 5-fold cross-validation with 50 repeats. Stability frequency scores of the selected OTUs were calculated (function perf) on the final sPLS-DA model with 5-fold cross-validation and 50 repetitions [30]. Individual samples were presented on a score plot and were distinguished by treatments with colour and 95% confidence ellipses. Discriminant genera were plotted according to their contribution weight to the component 1 of 2 of sPLS-DA and were distinguished by treatments with colours [31].

Finally, we tested the association between cecum microbiota and ADG of piglets of Low and High dose yeast supplemented groups using a Partial Least Squares Analysis (PLS) approaches and the network function [32]. The ‘network’ function calculated a similarity measure between X (OTUs) and Y (ADG values) variables in a pairwise manner. OTUs showing a pairwise associations with scores greater than 0.50 were considered significant.

**Results**

**Growth performance**

Age and treatment significantly influenced the piglet body weight ($P < 0.001$). There was no significant difference in body weight between the different treatment groups on day 1 of age.
Piglets that received Low or High dose yeast supplements were heavier than Control piglets by 10 days of age and remained heavier until the end of the study ($P < 0.05$) (Fig 2A).

The overall ADG was higher in both the High and Low dose yeast-treated piglets as compared to the Control piglets ($P = 0.017$ and $P = 0.016$, respectively) (Fig 2B). As there was no difference in performance between the High dose and Low dose of yeast treated piglets, we pooled the data for the low dose and high dose yeast groups and compared with the control group (Fig 2C and 2D). Yeast treatment significantly improved ($P < 0.05$) body weight and ADG.

Microbial diversity and species richness

Table 1 details the various microbial diversity indices and statistical differences among treatments. Number of observed OTUs and Chao richness estimate has indicated that the microbial
communities in the hind gut of the Control piglets were more rich in terms of bacterial species present than the community in the Yeast treated piglets ($P < 0.05$), while no difference was observed in Shannon diversity index. As shown in Table 1 and Fig 3, the lower dose yeast dietary supplementation reduced the number of observed OTUs and Chao’s richness estimate compared to Control ($P = 0.01$). No significant difference between the High dose yeast dietary supplementation and Control were reported for number of observed OTUs and alpha diversity indices.

**Microbial community structure**

Multivariate analyses showed treatment was significant in driving diversity assessed on Bray-Curtis Dissimilarity ($P = 0.03$). A significant difference was observed between Control and Low dose yeast supplemented group ($P = 0.02$) and between Low dose and High dose yeast supplemented groups ($P = 0.05$), while no difference was observed between Control and High dose yeast supplemented groups. NMDS plot based on the Bray-Curtis Dissimilarity did not show any distinct clustering pattern between the treatment groups. However, the Low dose yeast supplemented group showed a higher dispersion compared to the Control group (Fig 4). This difference was further supported by the permutational MANOVA test ($P = 0.02$).

**Phylogenetic assignment and discriminant bacterial population**

Phylum level classification of the bacterial population revealed that more than 90% of the bacteria in the hind gut of neonatal piglets belong to either one of the three major phyla Firmicutes, Bacteroidetes, and Actinobacteria (Fig 5A). There was no significant difference between the groups in the relative abundance of Firmicutes; 83.14% in Control, 82.04% in Low dose yeast, and 81.99% in High dose yeast group. The relative abundance of phylum Bacteroidetes tended to be lower in the Low dose yeast group (4.88%) than in Control group (14.12%) ($P\text{ adj.} = 0.06$) (Fig 5B), while no significant difference was reported for High dose yeast supplementation (8.39%) compared to Control (9.60%). In contrast a higher proportion of phylum Actinobacteria was reported in the Low dose yeast supplemented group than in Control group (12.01% in Low dose yeast, 2.73% in Control; $P\text{ adj.} = 0.03$), while no significant difference was reported between High dose yeast supplementation group (8.39%) and Control group (Fig 5B). At Family level, Ruminococcaceae (31.6%), followed by Lactobacillaceae (16.8%) and Erysipelotrichaceae (9.7%) were the most abundant and no significant difference was observed among groups.

At Genus level, Unclassified Ruminococcaceae (27.2%), followed by Lactobacillus (16.8%) and Unclassified Clostridiales (9.2%) were the most abundant. Table 2 reports the significantly different genera between yeast and Control groups and their relative abundance. A trend of
significant differences was reported for *Prevotella*, *Unclassified Lachnospiraceae*, *Oscillospira* and *Dorea* genera among groups after the FDR correction ($P_{adj.} < 0.1$). A higher number of different genera abundance can be described without the multiple corrections. Control had higher *Oscillospira* abundance than both Low and High yeast supplemented groups ($P < 0.05$), higher abundance of *Dorea*, *Prevotella*, *Unclassified Lachnospiraceae*, *Acidaminococcus* and *Unclassified Peptostreptococcaceae* than Low yeast supplemented group ($P < 0.05$) and higher abundance of *Veillonella* than High yeast supplemented group ($P < 0.05$). A trend of higher *Dorea* abundance was observed in Control than in High yeast group ($P = 0.08$). Low dose yeast group had higher *Collinsella* than the Control group ($P = 0.03$) and a trend of higher *Blautia*
(P = 0.07). High dose yeast group had higher Blautia (P = 0.007) than Control group and a trend of higher Pseudoramibacter_Eubacterium and Roseburia (P < 0.1).

The sPLS-DA was applied to identify specific genera that could potentially distinguish among the treatment groups. Fig 6A, 6B and 6C shows the individual score plot and the contribution plots, while Table 3 lists the discriminant genera for the different treatments. As can be seen in Fig 6A the final model resulted in clustering of samples according to treatment groups. The 1st and 2nd latent components contribute towards 9% and 10% of explained variance. The Control group was discriminated by a higher abundance of Veillonella, Dorea, Oscillospira, Clostridium and Prevotella genera; Low yeast dietary treatment group was discriminated by a higher abundance of Blautia and Eubacterium genera; High yeast dietary treatment group was discriminated by a higher abundance of Phascolarctobacterium, Anaerostipes, CF231, Parabacteroides, Eubacterium, Prevotella and Mogibacterium genera.

**Microbiota influence on piglet ADG**

Influence of microbiota composition on piglet ADG in the Low and High yeast supplemented groups were assessed using the PLS model. No OTUs were positively correlated with the
Fig 5. Microbiota differences at phylum level in cecum content of piglets treated with two different doses of yeast or water. Piglets were treated by oral gavage with either $5 \times 10^9$ cfu (Low) dose or $2.5 \times 10^{10}$ cfu (High) dose of live yeast or sterile water (Control) every other day starting from day one of age until weaning. Piglets were humanely euthanized on weaning day and Cecum content collected for microbiota analysis. A) Bar graphs show the mean relative abundance of each bacterial phyla calculated for each treatment group. B) Box plots show the proportion of the two abundant and significantly different bacterial Phyla among groups in the cecum microbiota of suckling piglets. Boxes displayed the interquartile range with line in the median and whiskers showed the minimal and maximal observed data. The number of piglets per group were respectively: 13 in Control; 15 in Low yeast; 16 in High yeast.

https://doi.org/10.1371/journal.pone.0219557.g005
piglets’ ADG in the Low dose yeast group (S2 Table). Thirteen OTUs were positively correlated with the piglets’ ADG (correlation > 0.50) in the High dose yeast group. Three out of thirteen OTUs belong to genus *Prevotella*, one OTU to genus *Blautia*. The remaining nine positively correlated OTUs were assigned only to the Family or Order taxonomy level; four OTUs, belong to Family *Ruminococcaceae* and three OTUs belong to Order *Clostridiales*, respectively. Three OTUs belonging to Order *Clostridiales*, Family *Erysipelotrichaceae* and Family *Ruminococcaceae* were negatively correlated with piglets ADG (Table 4).

**Discussion**

Results from this study revealed the impact of *S. cerevisiae* yeast supplementation on growth performance of suckling piglets and on the modulation of their cecum microbial composition. The effect of live yeast supplementation on the growth performance of pigs is controversial. No effect of yeast supplementation on body weight gain was reported in sows [4,33,34], neonatal pigs [35], and weanling pigs [5]. On the other hand, supplementation of pig diets with live yeast was reported to improve feed intake and weight gain in weanling pigs [7,8,9] and to counteract detrimental effect of ETEC or *Salmonella* infection in post-weaning piglets [10,11,12]. Similarly, live yeast supplementation to gestation and lactation sow diets has been shown to improve average daily weight gain of the piglets from yeast fed sows [36,37] possibly due to improved quality and increased level of IgG in colostrum and IgA in the sow milk [4,6], which may have also resulted in increased level of IgG in the serum of piglets due to passive immunity [34]. Our results add to and are in agreement with the later studies demonstrating improved growth rate of suckling piglets orally supplemented with live yeast. This is very important in practice, due to the positive correlation between the weight at weaning and the piglet survival [38]. The mode of action by which live yeast supplementation improves health and performance of pigs in general, and the sucking piglets in particular, is not completely clear. Although yeast components may have mediated a direct immune modulating effect ultimately contributing to improved health and performance [39,40,41]. Yeast supplementation...
and may also have induced changes in the gut microbial population which in turn mediated performance responses [42]. Indeed, the gut microbiota has been associated with modulation of host metabolism [43] and the host-microbiota interaction has been considered a frontier area for livestock science [44].

In our study, Low yeast supplementation had the greatest influence on the cecal microbiota profile of suckling piglets. The Low dose group had a reduced microbial richness compared with the Control group, indicating that live yeast supplementation can modulate the hind gut microbiota of suckling piglets by reducing its variability. Supporting our line of argument, previous studies conducted in suckling and weanling pigs have reported decreased total bacterial count and a reduced diversity of faecal microbial population in young pigs supplemented with live yeast as compared to control piglets [8,45]. In general, an increase of microbial diversity has been associated with a greater microbial ecosystem stability and with an improved health status of the host [46,47]. However, some studies disproved this correlation between biodiversity and ecosystem stability by introducing the concept that microbial ecosystem stability can...

Fig 6. Results of sPLS-DA for microbial diversity at Genus level in cecum content of piglets treated with two different doses of yeast or water. A) Individual score plot of the samples along the first two components. B, C) Contribution plot represented the contribution of each genus on the first and second component. Genus contribution ranked from bottom (most important) to top. Colors indicate the group in which the feature is most abundant. The number of piglets per group were respectively: 13 in Control; 15 in Low yeast; 16 in High yeast. 

https://doi.org/10.1371/journal.pone.0219557.g006
vary according to host-associated microbiomes upon different environment and stress response, supporting the concept that higher microbial variability does not always represent a favorable condition for the host [48,49]. During the first couple of weeks after birth, piglets have shown an unstable gut microbiota; the alpha diversity indices, which reflect the microbial variability, have been reported to increase markedly until weaning [46], and a stable adult-like

Table 3. Discriminant genera in cecum content of sucking piglets treated with two different doses of yeast or water. Results of genera contribution weight to the components 1 and 2 of sPLS-DA.

| Genus                | Value.var | Stability | PC | Treatment |
|----------------------|-----------|-----------|----|-----------|
| Veillonella          | 0.58      | 0.76      | 1  | Control   |
| Dorea                | 0.52      | 0.80      | 1  | Control   |
| Oscillospira         | 0.51      | 0.82      | 1  | Control   |
| Clostridium          | 0.28      | 0.45      | 1  | Control   |
| Blautia              | -0.25     | 0.52      | 1  | Low Yeast |
| Phascolarctobacterium| 0.45      | 0.83      | 2  | High Yeast|
| Collinsella          | -0.45     | 0.79      | 2  | Low Yeast |
| Anaerostipes         | 0.40      | 0.95      | 2  | High Yeast|
| Parabacteroides      | 0.35      | 0.71      | 2  | High Yeast|
| [Eubacterium]        | 0.26      | 0.74      | 2  | High Yeast|
| [Eubacterium]        | -0.24     | 0.70      | 2  | Low Yeast |
| [Prevotella]         | 0.13      | 0.53      | 2  | High Yeast|
| Mogibacterium        | 0.12      | 0.63      | 2  | High Yeast|
| Prevotella           | 0.11      | 0.44      | 2  | Control   |

1 Value.var, expresses the variance explained by the single genera.

2 Freq, express the frequencies by which the genera were chosen among the 100 repetitions of the cross validation.

3 PC stands for the principal component that discriminate the genera.

4 Treatment: Control = no yeast; Low yeast = 5 x 10^9 cfu; High yeast = 2.5 x 10^10 cfu of live S. cerevisiae per pig every other day.

The number of piglets per group were respectively: 13 in Control; 15 in Low yeast; 16 in High yeast.

https://doi.org/10.1371/journal.pone.0219557.t003

Table 4. Discriminant OTUs associate with piglets’ ADG in High dose yeast supplemented group.

| Pairwise association with ADG | OTUs          |
|------------------------------|---------------|
| 0.63                         | Genus Prevotella|
| 0.62                         | Genus Prevotella|
| 0.58                         | Order Clostridiales |
| 0.58                         | Order Clostridiales |
| 0.57                         | Order Clostridiales |
| 0.56                         | Family Ruminococcaceae |
| 0.55                         | Family Ruminococcaceae |
| 0.53                         | Genus Blautia |
| 0.53                         | Genus Prevotella |
| 0.53                         | Order Clostridiales |
| 0.52                         | Family Ruminococcaceae |
| 0.52                         | Genus Blautia |
| 0.51                         | Family Ruminococcaceae |
| -0.50                        | Order Clostridiales |
| -0.50                        | Family Erysipelotrichaceae |
| -0.55                        | Family Ruminococcaceae |

https://doi.org/10.1371/journal.pone.0219557.t004
microbiota is reached between 10 and 21 days after weaning [46,50,51,52]. Following the concept proposed by Glasl et al. [48] that the microbial stability can vary according to the host condition, we proposed here that live yeast supplementation to piglets during this neonatal period may help to stabilize the gut microbiota and establish a favorable microbial condition in young piglets. This is supported by the better growth performance of yeast treated groups compared to the Control group observed in this study. In fact, in the probiotic strategy it is expected that the probiotic will colonize the intestinal site of the host and, to reduce the pathogens’ development, resulting in reduced microbial variability, as already proposed by other researchers [53,54]. On the other hand, the highly diverse microbiota in the cecum of Control pigs observed in this study could indicate that the gut microbiota in the Control group was subjected to greater instability reflecting the wide variety of bacteria that the piglets were exposed to during suckling period that may have easily established in the gut of the Control pigs in the absence of the yeast probiotic. However further and more robust studies are needed to confirm our hypothesis.

We reported here that the dominant bacterial populations in the cecum of suckling pigs were those belonging to the Firmicutes and to the Bacteroidetes phyla. This is in agreement with previous studies conducted in faeces [46, 55] and colon [53] of suckling piglets, but, apart from that, our results have also shown that the third major dominant bacteria were those that belong to the phylum Actinobacteria. In a previous study, phyla Fusobacteria and Proteobacteria were reported as the third and fourth dominant phyla in the faecal and colon microbial profile of suckling pigs, while phyla Actinobacteria was reported at a lower level (about 0.6–1.5) [46,53]. However, this difference could be ascribed to the different intestinal tract analyzed among studies, indeed our finding on the abundance of Actinobacteria is in agreement with the results reported by Yu et al. (2018) in the cecum of suckling piglets [56]. In our study, a higher relative abundance in Actinobacteria was observed in the Low yeast group compared to control group as already reported by Kiros et al. (2018) [57] using the same yeast strain and dose and by Brousseau et al. (2015) [58] using the Saccharomyces cerevisiae subsp. boulardii along the hind-gut (cecum and colon) of post-weaning pigs. This may highlight that yeast supplementation selects the Actinobacteria both in pre and post-weaning phases. In addition, phylum Actinobacteria has been indicated as the most stable phylum in piglets’ faeces before and after weaning [46]. Having a higher relative abundance of this phyla during suckling period may favor its resiliency after weaning and it may contribute to a greater microbial stability during the weaning transition.

Furthermore, differences at genus level were observed among groups. The Low dose yeast group had a higher relative abundance of Blautia, Eubacterium and Collinsella, bacterial genera which are commonly present in mammalian intestinal microbiota. Previous findings on these genera were mainly related to human and mice and increased abundance of mentioned genera have been linked with good intestinal health of the host [57–63]. For example, Collinsella genus belongs to Actinobacteria phylum and has been previously reported to increase in colon and cecum of yeast-supplemented weaned piglets [57] and has been indicated as one of the major utilizer of lactose in human gut and can modulate the virulence and pathogenicity of potential enteric pathogens [59]. Blautia genera can ferment different type of carbohydrates to produce acetic acid, lactic acid and ethanol [60] and have been associated with an improvement in glucose metabolism in mice [61]. Eubacterium have been reported to produce butyric acid, which is particularly important for colonic homeostasis [62,63]. The divergent response of microbial composition to the yeast dosage has been previously reported [64,65], thus our observation emphasizes again the importance of probiotic dosage in influencing the microbial profile. Indeed, repeated large introductions of a probiotic strain does not always result in an increase of the probiotic effect in modulating the microbial profile [66]. In our study, it was
possible to discriminate specific genera which were influenced by the highest yeast dose. Similar to the Low yeast supplementation, the High dose yeast supplementation increased the relative abundance of genera associated with SCFAs production, including *Eubacterium*, *Anaerostipes* which are butyrate producers [63], *Parabacteroides* which are succinate producers and *Phascolarctobacterium* which produce propionate via succinate fermentation [67]. Therefore, it seems that both Low and High dose yeast supplementation improved the level of short chain fatty acid producing bacteria, which may help to promote the piglets’ intestinal health. However, our study did not measure the level of SCFAs in the cecum of the piglets and hence further studies are needed to verify this hypothesis and to associate oral yeast supplementation with modification of the microbial metabolism in piglets’ cecum.

Furthermore, since both the Low and High dose supplemented yeast group showed significantly higher ADG values compared to Control, and as ADG can be influenced by intestinal microbial metabolism, we tested the association between cecum microbial profile and the piglets’ performance. In the Low dose yeast, no positive correlation between cecum microbiota and piglets ADG has been observed, while in the High dose yeast group we observed that *Prevotella* was positively correlated with piglets’ ADG, in agreement with previous findings [68,69]. This result suggests that yeast supplementation may affect the piglets’ ADG through different mechanisms depending on the yeast dose and further elucidation would be needed to confirm this.

The positive correlation between *Prevotella* abundancy and the piglets’ growth performance has been attributed to its ability to process complex dietary saccharides of the diet and favoring monosaccharide uptake by the host [69]. In our study, piglets had no access to creep feed, however, bacteria from genus *Prevotella* can also use monosaccharide such as glucose which may results from the digestion of the milk-derived lactose, to produce SCFAs [70]. The production of SCFAs is one of the mechanisms by which intestinal microbiota can influence and promote the host metabolism and physiology [71] and may explain the positive correlation between *Prevotella* and growth performance observed in our study. Overall, piglets’ performance can be influenced also by sows’ conditions [72], piglet birth weight and litter size [73, 74], piglets feed intake [73], colostrum and milk composition [74, 75], factors which we were not able to control in the present study. Thus, further elucidation of the positive correlation between *Prevotella* and ADG of suckling piglets observed in our study is desirable.

**Conclusion**

In conclusion, our results showed that yeast supplementation during sucking period improved piglet performance and shaped the piglet cecum microbiota composition in a dose dependent way. The lower yeast tested dose was more efficient in modifying the cecal microbial profile reducing the bacterial richness in cecum. Furthermore, both lower and higher doses of yeast promoted the development of bacteria known as SCFAs producers, which may have positively influenced the piglet metabolism resulting in improved performance; however, measuring the level of SCFAs produced is needed to confirm this hypothesis. In addition to this, studies aimed at further elucidation of the mechanisms by which different dose of live yeast supplementation interacts with the host to improve piglet health and growth are desirable in order to promote yeast supplementation as an alternative strategy to increase piglets’ robustness before weaning with an ultimate objective of reducing the use of antibiotic in the swine industry.

**Supporting information**

S1 Table. Piglets’ weight at the different time points and piglets’ ADG. Piglets were treated by oral gavage with either 5 x 10⁹ cfu (Low) dose or 2.5 x 10¹⁰ cfu (High) dose of live yeast or...
sterile water (Control) every other day starting from day one of age until weaning. Body weight was recorded at regular intervals and presented either as piglet weight in kilograms or daily weight gain in kilograms.

(XLSX)

S2 Table. PLS results for discriminant OTUs associate with piglets’ ADG in Low dose yeast supplemented group.

(XLSX)

Acknowledgments

The authors would like to express their appreciation to all staff at the Prairie Swine Center Inc especially, Mr. Brian Andries (Manger-Operations) and Dr. Denise Beaulieu (research scientist) for facilitating all our animal experiments. Furthermore, the authors wanted to thanks Dr. Vincenzo Motta for his methodological advices in the microbiota bioinformatics analysis.

Author Contributions

Conceptualization: Tadele G. Kiros, Romain D’Inca, Eric Auclair, Andrew G. van Kessel.

Data curation: Diana Luise.

Formal analysis: Tadele G. Kiros, Diana Luise, Hooman Derakhshani, Renee Petri.

Funding acquisition: Andrew G. van Kessel.

Investigation: Tadele G. Kiros.

Project administration: Tadele G. Kiros, Romain D’Inca, Eric Auclair, Andrew G. van Kessel.

Resources: Andrew G. van Kessel.

Writing – original draft: Tadele G. Kiros, Diana Luise.

Writing – review & editing: Paolo Trevisi, Andrew G. van Kessel.

References

1. Moallem U, Lehrer H, Livshitz L, Zachut M, Yakoby S. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J. Dairy Sci. 2009; 92: 343–351. https://doi.org/10.3168/jds.2007-0839 PMID: 19109291

2. Desnoyers M, Gigler-Revardin S, Bertin G, Duvaux-Ponter C, Sauvant D. Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 2009; 92(4):1620–1632 https://doi.org/10.3168/jds.2008-1414 PMID: 19307644

3. Poppy GD, Rabiee AR, Lean IJ, Sanchez KV, Dorton KL, Morley PS. A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of Saccharomyces cerevisiae on milk production of lactating dairy cows. J. Dairy Sci. 2012; 95: 6027–6041. https://doi.org/10.3168/jds.2012-5577 PMID: 22921623

4. Jurgens MH, Rikabi RA, Zimmerman DR. The effect of dietary active dry yeast supplement on performance of sows during gestation-lactation and their pigs. J. Anim Sci. 1997; 75: 593–597. https://doi.org/10.2527/1997.753593x PMID: 9078472

5. Kornegay ET, Rheinwelker D, Lindemann MD, Wood CM. Performance and nutrient digestibility in weanling pigs as influenced by yeast culture additions to starter diets containing dried whey or one of 2 fiber sources. J Anim Sci. 1995; 73: 1381–1389. https://doi.org/10.2527/1995.7351381x PMID: 7665367

6. Zanello G, Meurens F, Serreau D, Chevaleyre C, Melo S, Berri M, et al. Effects of dietary yeast strains on immunoglobulin in colostrum and milk of sows. Vet Immunol Immunopathol. 2013; 152: 20–27. https://doi.org/10.1016/j.vetimm.2012.09.023 PMID: 23092748
Effect of *Saccharomyces cerevisiae* supplementation on performance and cecum microbiota of suckling piglets

7. Mathew AG, Chattin SE, Robbins CM, Golden DA. Effects of a direct-fed yeast culture on enteric microbial populations, fermentation acids, and performance of weanling pigs. J Anim Sci. 1998; 76: 2138–2145. https://doi.org/10.2527/1998.7682138x PMID: 9734864

8. van Heugten E, Funderburke DW, Dorton KL. Growth performance, nutrient digestibility, and fecal microflora in weanling pigs fed live yeast. J Anim Sci 2003; 81: 1004–1012. https://doi.org/10.2527/2003.811004x PMID: 12723090

9. Li J, Li D, Gong L, Ma Y, He Y, Zhai H. Effects of live yeast on the performance, nutrient digestibility, gastrointestinal microbiota and concentration of volatile fatty acids in weanling pigs. Arch AnimNutri. 2006; 60: 277–288.

10. Trevisi P, Colombo M, Priori D, Fontanesi L, Galimberti G, Calò G, et al. Comparison of three patterns of feed supplementation with live *Saccharomyces cerevisiae* yeast on post weaning diarrhea, health status, and blood metabolic profile of susceptible weaning pigs orally challenged with *Escherichia coli* F4ac. J Anim Sci. 2015; 93: 2225–2233. https://doi.org/10.2527/jas.2014-8539 PMID: 2620319

11. Trevisi P, Latorre R, Priori D, Luise D, Archetti I, Mazzoni M. et al. Effect of feed supplementation with live yeast on the intestinal transcriptomic profile of weaning pigs orally challenged with *Escherichia coli* F4. Animal. 2017; 11: 33–44. https://doi.org/10.1017/S1751731116001178 PMID: 27358089

12. Price KL, Totty HR, Lee HB, Ut MD, Fitzner GE, Yoon I, et al. Use of *Saccharomyces cerevisiae* fermentation product on growth performance and microbiota of weaned pigs during Salmonella infection. J Anim Sci. 2010; 88: 3896–3900. https://doi.org/10.2527/jas.2009-2726 PMID: 20656973

13. Zhang A, Lee WBD, Lee SK, Lee KW, An GH, Song HB, Lee CH. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Sci. 2005; 84: 1015–1021.

14. Paryad A, Mahmoudi M. Effect of different levels of supplemental yeast (*Saccharomyces cerevisiae*) on performance, blood constituents and carcass characteristics of broiler chicks. Afr J Agric Res. 2008; 3: 835–842.

15. Thompson CL, Holmes AJ. A window of environmental dependence is evident in multiple phylogenetically distinct subgroups in the faecal community of piglets. FEMS Microbiol Lett 2009; 290: 91–97 https://doi.org/10.1111/j.1574-6968.2008.01404.x PMID: 19016877

16. National Research Council. 2012. Nutrient requirements of swine: Eleventh Revised Edition. Washington, DC: The National Academies Press.

17. Dumonceaux TJ, Hill JE, Briggs SA, Amoako KK, Hemmingsen SM, Van Kessel AG. Enumeration of specific bacterial populations in complex intestinal communities using quantitative PCR based on the chaperonin-60 target. J Microbiol Methods. 2006; 64: 46–62. https://doi.org/10.1016/j.mimet.2005.04.006 PMID: 16112762

18. Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeehan T, Hagevoort RG, et al. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol. 2008; 8: 125. https://doi.org/10.1186/1471-2180-8-125 PMID: 1865285

19. Fan L, Kerensa ME, Torsten T. Reconstruction of ribosomal RNA genes from metagenomic data. PloS ONE. 2012, 7: e39948. https://doi.org/10.1371/journal.pone.0039948 PMID: 22761935

20. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Envir Microbiol. 2006; 72: 5069–5072.

21. Caporaso JG, BITtinger K, Bushman FD, DeSantis TZ, Andersen JL, Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 2010; 26: 266–267. https://doi.org/10.1093/bioinformatics/btp636 PMID: 19914921

22. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J. Mol. Biol. 1990; 215: 403–410. https://doi.org/10.1016/0022-2836(90)90320-L PMID: 2231712

23. Price MN, Dehal PS, Arkin AP. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One. 2010; 5: e9490. https://doi.org/10.1371/journal.pone.0009490 PMID: 20224823

24. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

25. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, et al. Quality-filtering vastly improves diversity estimates from illumina amplicon sequencing. Nat methods. 2013; 10, 57–59. https://doi.org/10.1038/nmeth.2276 PMID: 23202435

26. McMurdie PJ, Holmes S, KIndt R, Legendre P, O’Hara R. PhylSeq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. 2013; 8, e61217. https://doi.org/10.1371/journal.pone.0061217 PMID: 23630581

27. Dixon P. VEGAN, a package of R functions for community ecology. J of Vegetat Sci. 2003; 14, 927–930.
28. Rohart F, Gautier B, Singh A, Lê Cao KA. mixOmics: An R package for ‘omics feature selection and multiple data integration. PLoS Comput Biol. 2017; 13(11): e1005752. https://doi.org/10.1371/journal.pcbi.1005752 PMID: 29099853

29. Chao A. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of statistics. 1984, 11: 265–70.

30. Lê Cao KA, Martin PGP, Robert-Granié C, Besse P. Sparse canonical methods for biological data integration: Application to a cross-platform study. BMC Bioinformatics. 2009; 10: 1–17.

31. Lê Cao KA, Boitard S, Besse P. Sparse PLS discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. BMC Bioinformatics. 2011; 12: 253. https://doi.org/10.1186/1471-2105-12-253 PMID: 21693065

32. Abdi H (2010). Partial least squares regression and projection on latent structure regression (PLS Regression). Wiley Interdisciplinary Reviews: Computational Statistics, 2(1), 97–106.

33. Veum TL, Reyes J, Ellersieck M. Effect of supplemental yeast culture in sow gestation and lactation diets on apparent nutrient digestibilities and reproductive-performance through one reproductive-cycle. J Anim Sci. 1995; 73: 1741–1745. https://doi.org/10.2527/1995.7361741x PMID: 7673068

34. Jang YD, Kang KW, Piao LG, Jeong TS, Auclair E, Jonvel S, et al. Effects of live yeast supplementation to gestation and lactation diets on reproductive performance, immunological parameters and milk composition in sows. Livestock Sci. 2013; 152: 167–173.

35. Veum TL, Bowman GL. Saccharomyces Cervisiae yeast culture in diets for mechanically-fed neonatal piglets and early growing self-fed pigs. J Anim Sci. 1973; 37: 67–71.

36. Kim SW, Brandtherr M, Freeland M, Newton B, Cook D, Yoon I. Effects of yeast culture supplementation to gestation and lactation diets on growth of nursing piglets. Asian-Australasian J Anim Sci. 2008; 21: 1011–1014.

37. Shen YB, Carroll JA, Yoon I, Mateo RD, Kim SV. Effects of supplementing Saccharomyces cerevisiae fermentation product in sow diets on performance of sows and nursing piglets. J Anim Sci. 2011; 89: 2462–2471. https://doi.org/10.2527/jas.2010-3642 PMID: 21383042

38. López-Vergé S, Gasa J, Farré M, Coma J, Bonet J, Solá-Oriol D. Potential risk factors related to pig body weight variability from birth to slaughter in commercial conditions. Transl. Anim. Sci. 2018; 2: 383–395.

39. Jiang Z, Wei S, Wang Z, Zhu C, Hu S, Zheng C et al. Effects of different forms of yeast Saccharomyces cerevisiae on growth performance, intestinal development, and systemic immunity in early-weaned piglets. J Anim Scie Biot, 2015; 6: 47.

40. Zanello G, Berri M, Dupont J, Sizaret PY, D’Inca R, Salmon H, et al. Saccharomyces cerevisiae modulates immune gene expressions and inhibits ETEC-mediated ERK1/2 and p38 signaling pathways in intestinal epithelial cells. PLoS One. 2011; 6: e18573. https://doi.org/10.1371/journal.pone.0018573 PMID: 21483702

41. Shen YB, Piao XS, Kim SW, Wang L, Liu P, Yoon I, et al. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J Anim Sci. 2009; 87: 2614–2624. https://doi.org/10.2527/jas.2008-1512 PMID: 19395514

42. More MI, Swidsinski A. Saccharomyces boulardii NCIMB 1-745 supports regeneration of the intestinal microbiota after diarrheic dysbiosis—a review. Clin Exp Gastroenterol. 2015; 11: 237–255.

43. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012; 489: 242. https://doi.org/10.1038/nature11552 PMID: 22972297

44. Stokes CR. The development and role of microbial-host interactions in gut mucosal immune development. J Anim Sci Biotechnol. 2017; 8:12. https://doi.org/10.1186/s40104-016-0138-0 PMID: 28149511

45. Upadrashta A, O’Sullivan L, O’Sullivan O, Sexton N, Lawlor PG, Hill C, et al. The Effect of dietary supplementation with spent cider yeast on the swine distal gut microbiome. PLoS One 2013; 8: e75714. https://doi.org/10.1371/journal.pone.0075714 PMID: 23410736

46. Chen L, Xu Y, Chen X, Fang C, Zhao L and Chen F. The maturing development of gut microbiota in commercial piglets during the weaning transition. Front Microbiol 2017; 8: 1688. https://doi.org/10.3389/fmicb.2017.01688 PMID: 28928724

47. Konopka A. What is microbial community ecology? ISME J. 2009; 3: 1223–1230. https://doi.org/10.1038/ismej.2009.88 PMID: 19657372

48. Glasi B, Smith CE, Bourne DG, Webster NS. Exploring the diversity-stability paradigm using sponge microbial communities. Sci Reports. 2018; 8: 8425.

49. Falony G, Vieira-silva S, Raes J. Richness and ecosystem development across faecal snapshots of the gut microbiota. Nat Microbiol. 2018; 3:526–8. https://doi.org/10.1038/s41564-018-0143-5 PMID: 29693658

50. Tatem AO, Pearson NJ. The gut microbiota in human health and disease: a review. Front Microbiol 2012; 3: 148. https://doi.org/10.3389/fmicb.2012.00148 PMID: 22523202
50. Thompson CL, Wang B, Holmes AJ. The immediate environment during postnatal development has long-term impact on gut community structure in pigs. ISME J. 2008; 2: 739–748. https://doi.org/10.1038/ismej.2008.29 PMID: 18356821

51. Petri D, Hill JE, Van Kessel AG. Microbial succession in the gastrointestinal tract (GIT) of the pre-weaned pig. Livestock Sci. 2010; 133: 107–109.

52. Motta V, Luise D, Bosi P, Trevisi P. Faecal microbiota shift during weaning transition in piglets and evaluation of AO blood types as shaping factor for the bacterial community profile. PLoS ONE 2019; 14(5): e0217001. https://doi.org/10.1371/journal.pone.0217001 PMID: 31095619

53. Poulsen AR, De Jonge N, Nielsen JL, Ole H, Lauridsen C, Cutting SM, et al. Impact of Bacillus spp. spores and gentamicin on the gastrointestinal microbiota of suckling and newly weaned piglets. PLoS ONE 2018; 13:e0207382. https://doi.org/10.1371/journal.pone.0207382 PMID: 30481191

54. Wang JO, Yin FG, Zhu C, Yu H, Niven SJ, de Lange CFM, et al. Evaluation of probiotic bacteria for their effects on the growth performance and intestinal microbiota of newly-weaned pigs fed fermented high-moisture maize. Livest Sci. 2012; 145(1–3):79–86

55. Xiao L, Estellé J, Killierich P, Ramayo-Caldas Y, Xia Z, Feng Q, et al. A reference gene catalogue of the pig gut microbiome. Nat Microbiol. 2016; 6161.

56. Yu M, Mu C, Zhang C, Yang Y, Su Y. Marked response in microbial community and metabolism in the ileum and cecum of piglets after early antibiotics exposure. Front Microbiol. 2018; 9:1166. https://doi.org/10.3389/fmicb.2018.01166 PMID: 29899739

57. Kiros TG, Derakhshani H, Pinloche E, D’Inca R, Marshall J, Auclair E, et al. Effect of live yeast Saccharomyces cerevisiae (Actisaf Sc 47) supplementation on the performance and hindgut microbiota composition of weaning pigs. Sci Reports. 2018; 8: 1–13.

58. Brousseau J, Talbot G, Beaudoin F, Lauzon K, Roy D, Lessard M. Effects of probiotics Pedicoccus acidilactici strain MA18 / 5M and Saccharomyces cerevisiae subsp. boulardi strain SB-CNMC 1-1079 on fecal and intestinal microbiota of nursing and weanling piglets. J Anim Sci. 2015; 93:5313–5326. https://doi.org/10.2527/jas.2015-9190 PMID: 26641051

59. Bag S, Ghosh TS, Das B. Complete genome sequence of Collinsella aerofaciens isolated from the gut of a healthy Indian subject. Genome Announc. 2017; 5: e01361–17. https://doi.org/10.1128/genomeA.01361-17 PMID: 29167267

60. Liu C, Finegold SM, Song Y, Lawson PA. Reclassification of Clostridium coccoidei, Ruminococcus hansenii, Ruminococcus hydrogenotrophicus, Ruminococcus luti, Ruminococcus productus and Ruminococcus schinkii as Blautia coccoidei gen. nov., comb. nov., Blautia hansenii comb. nov., Blautia hydrogenotrophica comb. nov., Blautia luti comb. nov., Blautia producta comb. nov., Blautia schinkii comb. nov. and description of Blautia wexlerae sp. nov., isolated from human faeces. Int J Syst Evol Microbiol. 2008; 58: 1986–1902. https://doi.org/10.1099/ijs.0.65208-0 PMID: 18676476

61. Yang J, Bindels LB, Munoz RRS, Martinez I, Walter J, Ramer-Tait AE, et al. Disparate metabolic responses in mice fed a high-fat diet supplemented with maize-derived non-digestible feruloylated oligo-galloyl polycosaccharides are linked to changes in the gut microbiota. PLoS ONE. 2016; 11: e0146144. https://doi.org/10.1371/journal.pone.0146144 PMID: 26731528

62. Trachsel J, Bayles DO, Looft T, Levine UY, Allen HK. Function and phylogeny of bacterial butyryl coenzyme A: acetate transferases and their diversity in the proximal colonic swine. Appl Env Microbiol. 2016; 82: 6788–6798.

63. Levine UY, Looft T, Allen HK, Stanton TB. Butyrate-producing bacteria, including mucin degraders, from the swine intestinal tract. Appl Env Microbiol. 2013; 79: 3879–3881.

64. Jiang Y, Ogunade IM, Qi S, Hackmann TJ, Staples CR, Adesogan AT. Effects of the dose and viability of yeast Saccharomyces cerevisiae and Ruminococcus mucilaginosus strains SB-CNCM I-1079 and SB-CNMC I-1079 on performance and gut microbiota of newly-weaned piglets. J Anim Sci. 2016; 82: 6788–6798. https://doi.org/10.1038/ismej.2008.29 PMID: 18356821

65. Thompson CL, Wang B, Holmes AJ. The immediate environment during postnatal development has long-term impact on gut community structure in pigs. ISME J. 2008; 2: 739–748. https://doi.org/10.1038/ismej.2008.29 PMID: 18356821

66. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. Nature. 2017; 548, 43–51. https://doi.org/10.1038/nature22922 PMID: 28770836

67. Wu F, Guo X, Zhang J, Zhang M, Ou Z, Peng Y. Phascolarctobacterium faecium abundant colonization in human gastrointestinal tract. Exp Ther Med. 2017; 14: 3122–3126. https://doi.org/10.3892/etm.2017.4678 PMID: 28912861

68. Ramayo-Caldas Y, Mach N, Lepage P, Levenez F, Denis C, Lemonnier G, et al. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. ISME J. 2016; 10: 2973–2977. https://doi.org/10.1038/ismej.2016.77 PMID: 27177190
69. Mach N, Berri M, Estellé J, Levenez F, Lemonnier G, Denis C, et al. Early-life establishment of the swine gut microbiome and impact on host phenotypes. Env Microb Reports. 2015; 7: 554–569.

70. Takahashi N. and Yamada T. Glucose metabolism by Prevotella intermedia and Prevotella nigrescens. Oral Microbiol Immunol. 2000; 15: 188–195. PMID: 11154402

71. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci. 2008; 105, 16767–16772. https://doi.org/10.1073/pnas.0808567105 PMID: 18931303

72. Vallet JL, Calderon-Diaz JA, Stalder KJ, Phillips C, Cushman RA, Miles JR, et al. Litter of origin trait effects on gilt development. J Anim Sci. 2016; 94, 96–105 https://doi.org/10.2527/jas.2015-9644 PMID: 26812316

73. Decaluwé R, Maes D, Wuyts B, Cools A, Piepers S, Janssens GPJ. Piglets' colostrum intake associates with daily weight gain and survival until weaning. Livest Sci. 2014; 162, 185–92

74. Picone G, Zappaterra M, Luise D, Trimigno A, Capozzi F, Motta V, et al. Metabolomics characterization of colostrum in three sow breeds and its influences on piglets' survival and litter growth rates. J Anim Sci Biotechnol. 2018; 9, 23 https://doi.org/10.1186/s40104-018-0237-1 PMID: 29527304

75. Leonard SG, Sweeney T, Bahar B, Lynch BP, O’doherty JV. Effect of maternal fish oil and seaweed extract supplementation on colostrum and milk composition, humoral immune response, and performance of suckled piglets. J Anim Sci. 2010; 88(9), 2988–2997.