# Effect of live yeast supplementation in sow diet during gestation and lactation on sow and piglet fecal microbiota, health, and performance

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Running title: Yeast supplementation in sow diet and piglet performance

# Effect of live yeast supplementation in sow diet during gestation and lactation on sow and piglet fecal microbiota, health and performance[1]

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## Lay Summary

Feeding live yeast *Saccharomyces cerevisiae var. boulardii* (**SB**) in pig diets is recommended to promote a better health and reduce antibiotic use during critical periods like weaning. Our study was conducted to determine if SB added in the diet of sows during the last 2 mo of gestation and the 4 wk of lactation may contribute to supporting health and performance of their piglets before and after weaning. We hypothesized that live SB supplementation to the sows may help improve the health and metabolic status of the sows, and consequently the quality of milk and microbiota provided to the piglets. Supplementation of sow diet with SB during gestation and lactation induced modifications in the fecal microbiota of sows and their piglets. For piglets, the effects of SB fed to their mother were still observed 5 days after weaning. These modifications were however associated with changes neither in piglet ability to cope with the stress of weaning, nor in milk nutritional and immune composition.

## Teaser Text

Feeding sows with *Saccharomyces cerevisiae var. boulardii* during gestation and lactation impacted the fecal microbiota of piglets up to weaning but changed neither performance, nor health of piglets around weaning.

**Abstract**

Feeding probiotics like live yeast *Saccharomyces cerevisiae var. boulardii* (**SB**) in pig diets has been suggested to preserve health and reduce antibiotic use during critical periods like weaning. This study was conducted to determine whether SB added in the diet of sows during the last 2 mo of gestation and the 4 wk of lactation may contribute to supporting health and performance of piglets before and after weaning through changes in sow physiology, milk composition and fecal microbiota. Crossbred sows (n=45) from parity 1 to 9 were allocated to two dietary treatments, Control (n=23) and SB (n=22). Sows in the SB group were fed the same standard gestation then lactation diet as the Control sows but with the addition of SB at $1x10^9$ colony forming units/kg of feed. Piglets were weaned under challenging conditions consisting in mixing of litters, no pen cleaning and a 2-h period of non-optimal temperature exposure. Blood and feces were collected from sows on d 28 and 113 of gestation and d 6 (feces only) and 28 of lactation, and from piglets on d 6 (feces) and 28 of lactation and d 5 after weaning. Colostrum was collected during parturition and milk on d 6 of lactation. Supplementation of sow diets with SB influenced the fecal microbiota of the sows and their piglets. Five days after weaning, the alpha-diversity was lower ($P < 0.05$) in piglets from SB sows than in piglets from Control sows. Analysis of microbiota with Partial Least Square Discriminant Analysis discriminated feces from SB sows from that of Control sows at 110 d of gestation (29.4% error rate). Piglet feces could also be discriminated according to the diet of their mother, with a better discrimination early after birth (d 6 of lactation) than after weaning (d 5 post-weaning, 3.4% vs 12.7% error rate). Five d after weaning, piglets had greater white blood cell count, plasma haptoglobin concentration, and oxidative stress than

before weaning ($P$ <0.001). Nevertheless, SB supplementation in sow diets had no effect ($P$ > 0.05) on most of health criteria measured in blood and growth performance of piglets during lactation and the post-weaning period. Moreover, dietary supplementation of SB to sows did not elicit any changes ($P$ > 0.05) in their reproductive performance, metabolic and health status, nor in the immunoglobulin and nutrient concentration of colostrum and milk. In the present experimental conditions, feeding SB to sows influenced sow and piglet microbiota with no consequences on their health and performance.

**Key words:**

colostrum, milk, probiotic, *Saccharomyces cerevisiae boulardii*, weaning

**Abbreviations:** ADFI, average daily feed intake; ADG, average daily gain; BAP, biological antioxidant power; BW, body weight; CFU, colony forming units; dROM, reactive oxygen metabolites-derived compounds; FCR, feed conversion ratio; FFA, free fatty acid; G28, d 28 of gestation; G110, d 110 of gestation; G113, d 113 of gestation; IgG, immunoglobulins G; IgA, immunoglobulins A; L0, d 0 of lactation; L6, d 6 of lactation; L28, d 28 of lactation; OTU, operational taxonomic unit; PLS-DA, partial least square discriminant analysis; SB, *Saccharomyces cerevisiae boulardii*; sPLS, sparse partial least squares regression; W5, d 5 after weaning; W35, d 35 after weaning.

## INTRODUCTION

In most pig farms, piglets are usually weaned between 3 and 5 wk of age, an age at which their immune and digestive systems are still immature (Lallès et al., 2007; Campbell et al., 2013). At weaning, piglets cope with dietary change, separation from the sow, new environment and counterparts. All these changes cause a transient decrease in feed consumption, intestinal inflammation, and unbalanced and dysbiotic gut microbiota (Pie et al., 2004) and are risk factors for enteric disease and diarrhea (Gresse et al., 2017). Antibiotics have been overused for a long time to prevent and treat any kind of digestive disorders occurring after weaning regardless of their origin, bacterial or not. To face the risk of spreading of antibiotic resistance, biotechnical tools, such as biosecurity, vaccine, feeding strategies and additives, have been successfully implemented at weaning to improve digestive and immune capacities of weaned piglets (Kil and Stein, 2010; Heo et al., 2013).

The proper development and growth of piglets during lactation depend mostly on the sow and are determinant to strengthen the capacity of piglets to cope with the challenge of weaning (Blavi et al., 2021). Nutritional strategies applied to sows during gestation and lactation may improve the metabolic and health status of the sows as well as the immune and nutritional quality of their colostrum and milk they transfer to their piglets (Quesnel and Farmer, 2019) and might be relevant to improve health and performance of their litter before weaning. Feed supplementation with live yeast like *Saccharomyces cerevisiae* (**SB**) during late gestation and lactation has been shown to increase sow voluntary feed intake during lactation and litter weight at weaning (Tan et al., 2015; Domingos et al., 2021; Sun et al., 2021). Furthermore, positive effects of SB supplementation in feed of the sows were reported on milk production

(Domingos et al., 2021) and on colostrum immunoglobulin A and G contents (Guillou et al., 2012).

For a decade, there is an increasing interest to consider the microbiota as a major determinant of health and development of piglets before and after weaning (Gresse et al., 2017; Guevara et al., 2019). During the early stage of life, the digestive microbiota of piglets grows and matures in connection with its neonatal environment and the sow is probably a main vector of early colonization of the gut of its progeny. Feed supplementation with SB has been successfully used in nursed or weaned piglets to modulate digestive microbiota (Daudelin et al., 2011; Brousseau et al., 2015). However, there is no data describing the influence of SB in sow diet on the digestive microbiota of the piglets.

The main objective of this study was to determine whether live SB added in the diet of sows during gestation and lactation may contribute to support the health and performance of the piglets around weaning. Our hypothesis was that live SB supplementation to the sows may help improve the health and metabolic status of the sows, and consequently the quality of milk and microbiota provided to the piglets. To our knowledge, our study is the first one reporting conjointly the effects of SB supplementation in sow diet on sow and piglet performance, physiology and microbiota.

## MATERIALS AND METHODS

The experiment was carried out at INRAE (UE3P, Saint-Gilles, France), in compliance with the Directive 2010/63/UE on animal experimentation. The experimental protocol was approved by the regional Ethics Committee in Animal Experiment of Rennes (France) and by

130 the French Ministry of Higher Education, Research and Innovation (authorization
131 APAFIS#11015-2017080716549316).
132
133 ***Animals and Experimental Design***
134 To test our hypothesis on an adequate number of piglets at weaning, forty-eight Landrace x
135 Large White sows from parity 1 to 9 and their litter were used in 4 batches of 12 females.
136 Sows were inseminated with semen from Piétrain boars. At 28 d of gestation (**G28**), sows
137 were distributed into 2 dietary treatments, **Control** and **SB.** Sows in the SB group were fed
138 the same standard gestation then lactation diet as the Control sows but with the addition of
139 *Saccharomyces cerevisiae var. boulardii* CNCM I-1079 (Levucell SB®; Lallemand SAS,
140 France) as live yeast cells (minimum concentration of $1x10^{10}$ colony-forming unit (**CFU**/g)
141 added at 100 g/ton ($1x10^{9}$ CFU/kg of feed). The inclusion level was chosen according to the
142 manufacturer recommendations when SB is supplied during gestation and lactation. Parities
143 were balanced across treatments and batches.
144 From G28, sows were housed in groups of 6 in a pen with concrete floor (5 x 3.5 m) covered
145 with wood hulls. Cleaning and replenishment of bedding were done 4 times a week. The room
146 was equipped with individual feeding stalls and cup drinkers. Sows in the same pen were fed
147 the same experimental diet, SB or Control. At 106 d of gestation (G106), sows were moved to
148 the farrowing room and were kept in individual farrowing crates (1.77 x 2.4 m) thereafter.
149 The floor of the farrowing pens was made of slatted plastic. The farrowing crates were
150 equipped with 2 infrared heat bulbs. The ambient temperature was kept between 18 and 24 °C
151 in the gestation rooms while it was kept between 24 and 25 °C in the lactation rooms.

During gestation, and until the day of farrowing, sows were fed a conventional gestation diet (as-fed basis: 9.62 $MJ.kg^{-1}$ net energy, 13.6% crude protein, 0.5% digestible lysine, and 5.0% crude fiber, supplementary table 1). Feed allocation depended on sow body condition and backfat thickness and was between 2.0 and 2.2 kg/d, 2.5 and 2.7 kg/d, and 2.8 and 3.2 kg/d in early, mid, and late gestation (d 0 to 35, 36 to 80, and 81 to the day of farrowing, respectively). Feed was provided in 2 equal meals at 0900 h and 1500 h. From d 1 of lactation (d 0 of lactation being the day of farrowing), sows were fed a conventional lactation diet providing 9.80 $MJ.kg^{-1}$ net energy, 16.5% crude protein, 0.8% digestible lysine and 3.9% crude fiber (as-fed basis). They received between 2.7 and 3.3 kg on d 1 and then feed allowance was increased by 1 kg/d until *ad libitum* feeding, which was reached approximately on d 4 or 5 of lactation. During *ad libitum* feeding, feed troughs were filled 3 times a day, so that feed was always available. From G106 and throughout lactation, feed refusals were weighed daily, and actual feed intakes were calculated. Water was available *ad libitum* throughout the experiment.

Farrowing was induced by an intramuscular injection of prostaglandin F2α (2 mL of Dinolytic, Zoetis, France) at 114 d of gestation. Usual farm practices were performed for newly farrowed piglets, i.e., individual identification by tagging, iron injection, tail docking, and castration. Cross-fostering, if needed, was performed intra-treatment within 2 d after birth and only for male piglets because female piglets were used for blood and feces sampling. The piglets were offered a conventional prestarter feed (providing as-fed basis: 10.5 $MJ.kg^{-1}$ net energy, 19.2% crude protein, 1.3% digestible lysine, 3.0% crude fiber) during the fourth week of lactation. They were weaned at 28 d of lactation (**L28**) and vaccinated against porcine type

2 circovirus (PCV2) and *Mycoplasma hyopneumoniae* (Porcilis PCV M Hyo, MSD Santé Animale, France). No antibiotics were preventively provided through the diet or water.

At weaning, piglets were transferred into a postweaning unit and group-housed in pens of 9 to 11 piglets. Each pen housed piglets from 1 experimental treatment only (Control or SB). Because weaning conditions may be more challenging in commercial farms than in experimental units, piglets were weaned in challenging conditions. For that purpose, piglets with similar range of body weight (**BW**) from at least 4 litters were mixed in the same pen. Piglets were assigned to a pen using the weaning weight as main factor, and litter as second factor. Moreover, piglets were transferred into pens that were not cleaned after the departure of the previous batch. Lastly, the ambient temperature was set at 24 °C at the pig arrival in the postweaning building before being progressively increased until 28 °C in 4 to 6 h. The piglets stayed in the postweaning facilities until d 35 after weaning (**W35**) that corresponded to the end of the experiment. They were offered the prestarter feed for the first 5 d and then the starter diet until W35, with a 3 d-transition period between the 2 diets. The starter diet provided 9.3 $MJ.kg^{-1}$ net energy, 17.8% crude protein, 1.07% digestible lysine, 2.9% crude fiber as-fed basis (Supplementary Table 2). Feed and water were available ad libitum during this period.

***Measurements on Animals***

Sow BW and backfat thickness were recorded on G28, G106 and L28. Sows were also weighed just after parturition (**L0**). Backfat thickness was measured ultrasonically at the P2 site of the sow on both left and right flanks 6.5 mm away from the spine. All piglets were weighed and identified within 24 h after birth. Piglets were weighed on d 6 of lactation, L28

196 and the last day of the post-weaning period (W35). The date and most probable cause of piglet
197 death were recorded daily. After weaning, feed refusals were daily recorded at the pen level to
198 estimate feed intake.
199
200 ***Blood Sampling and Plasma Analyses***
201 Blood samples were collected by jugular vein puncture from all sows after an overnight
202 fasting on G28 before SB supplementation started, **G113** (i.e., d 113 of gestation**,** the term
203 being around 115 d of gestation), and L28 before piglets were weaned. Blood was collected
204 from 2 females per litter on L28 and at 5 d after weaning (**W5**). Females were chosen with
205 birth weights closest to the average birth weight of the litter. Samples (9 mL for sows and 4
206 mL for piglets) were collected from the jugular vein into vacutainers containing EDTA (for
207 blood cell formula and haptoglobin analyses) or heparin (for other parameters). Collection
208 period was limited to 2 min from the restraining of the sows to limit excessive stress and pain.
209 Piglets were manually maintained on the back during blood collection.
210 Whole blood from the EDTA tubes was immediately analyzed for blood cell count using an
211 automatic cell counter MS 9.5 (Melet Schloesing Laboratories, Osny, France). Samples from
212 both anti-coagulants were then centrifuged immediately at 3,000 x *g* at 4°C for 15 min.
213 Plasma samples were frozen at either -20°C or -80°C. Concentrations of glucose, lactate, free
214 fatty acids (**FFA**), creatinine, and urea were determined in sow plasma using an automated
215 colorimetric analyzer, Konelab$^{TM}$ 20i (Thermo Fisher Scientific Inc., Courtaboeuf, France)
216 and commercial kits (from Thermo Fisher Scientific, Vantaa, Finland, references 981304,
217 981811, and 981818 for glucose, creatinine, and urea; Horiba ABX SAS, Montpellier, France,

reference A11A01721 for lactate; and Sobodia, Montbonnot, France, references W1W434-91795 and W1W436-91995 for FFA). Other parameters were analyzed in plasma of sows and piglets. Haptoglobin, an acute phase protein used as an indicator of inflammatory status, was assayed by using a commercial kit (TP-801, Tridelta Ltd, Maynooth, Ireland). Hydroperoxides (**dROM**) and antioxidant capacity of plasma (Biological Antioxidant Power test, **BAP**) were quantified using commercial kits (references MC-003 and MC-437, Diacron, Grosseto, Italy). The intra-assay CV was 10% for haptoglobin, 6% for dROM and 2% for BAP.

***Feces Sampling and Scoring***

Feces were collected from sows on G28, **G110** (d 110 of gestation), L6, and L28 and from piglets on L6, L28, and W5 after rectal stimulation. Feces were collected from all sows and from 3 female piglets per litter. These piglets were those selected for blood sampling and a third one whom birth weight was also close to the average within-litter birth weight. Fecal samples were immediately frozen in liquid nitrogen, and then stored at -80 °C. After weaning, piglet feces consistency was scored daily at the pen level using a scale of 0 to 2: 0 for normal or solid feces; 1 for soft feces and 2 for liquid feces or diarrhea.

***Milk and Colostrum Sampling and Analyses***

Colostrum was collected between 1 and 2 h after the birth of the first piglet while milk was collected on L6. For milk collection, piglets were isolated from the sow for 45 min before collection and 20 IU of oxytocin (Ocytovem®, CEVA Santé Animale) were injected

intramuscularly 10 min before collection. Around 60-70 mL of colostrum and milk were collected by manual collection from all functioning teats. Samples were immediately filtered through a gauze and stored at -20°C. Dry matter, ash, protein, fat, lactose, and gross energy were assayed as previously described by Loisel et al. (2013). Immunoglobulins G (**IgG**) and A (**IgA**) were assayed in triplicate, IgG and IgA were assayed in colostrum while only IgA were assayed in milk. Both IgG and IgA were analyzed by ELISA using commercial quantification kits for porcine IgG and IgA (references A100-104 and A100-102, respectively, Bethyl Laboratories, Montgomery, Texas, USA). The intra and interassay CV were, respectively, 2.9 and 7.0% for IgG and 4.0 and 5.8% for IgA.

***Fecal Microbiota Analyses***

All fecal samples from sows were analyzed. For some litters on L6, only 2 piglets could be sampled due to the difficulty to collect feces at this young age. Fecal samples from 2 out of 4 batches of piglets were analyzed because of technical issues. Microbial DNA was extracted from 40-60 mg of feces using ZR-96 Soil Microbe DNA Kit (Zymo Research, Freiburg, Germany) according to the manufacturer's instruction. A 15 min bead beating step at 30 Hz was applied using a Retsch MM400 Mixer Mill. The V3 and V4 hypervariable regions of the 16S rRNA gene were amplified using the primers F343 (CTTTCCCTACACGACGCTCTTCCGATCTACGGRAGGCAGCAG) and R784 (GGAGTTCAGACGTGTGCTCTTCCGATCTTACCAGGGTATCTAATCCT). High-throughput sequencing was performed on a MiSeq sequencer using the Reagent Kit v3, according to the manufacturer's instruction (Illumina Inc., San Diego, CA) in the Genomic

and Transcriptomic Platform (INRAE, Toulouse, France) and as previously described (Drouilhet et al., 2016). Sequences were deposited in Sequence Read Archive: accession number is PRJNA821692. Extracted DNA samples that failed to be amplified were not submitted to sequencing and excluded.

Generated paired-end 250 bp sequences were assembled using Flash software, with 10bp minimum overlap and 10% maximum mismatch (Magoč et al., 2011). Assembled sequences were processed using FROGS pipeline (Escudié et al., 2018). First, sequences were preprocessed: cutadapt was used to remove sequences in which the two primers were not present, with a 10% tolerated mismatch, and to trim the primers (Martin, 2011), sequences between 350 and 480bp and without ambiguous base were kept. Preprocessed sequences were then clustered in Operational Taxonomic Units (OTUs) using SWARM algorithm (Mahé et al., 2014). Chimeric sequences detected by samples using UCHIME algorithm (Edgard et al., 2011), as well as singletons (i.e., OTU represented by only 1 read) were removed from all samples. A rarefaction step (12,183 reads kept per samples) was then applied. Taxonomic annotation of the OTUs was performed using the SILVA SSU Ref NR 132 database (Glöckner et al., 2017) and BLAST+ (Qi et al., 2005) and RDP (Wang et al., 2007) algorithms. BLAST hits with identity and coverage alignments higher than 99% were kept for annotation. Otherwise, species were annotated as unknown and RDP classifier results were used for higher rank. Bootstrap thresholds were set to 0.9 and 0.8 respectively for annotation at the genus rank and higher ranks. The alpha-diversity, i.e., the diversity within sample, was estimated using richness and Shannon index calculated with Vegan R package.

### *Statistical Analyses*

Data, except for mortality rate and piglet fecal scoring, were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). For sow and litter data, the sow or the litter represented the experimental unit. For sow performance, milk composition, and litter performance during lactation, the model included treatment (Control or SB), sow parity (primiparous or multiparous) and the interaction as main effects, and the batch (1, 2, 3, or 4) as random. After weaning, pig BW, average daily gain (**ADG**), and feed intake at the pen level were analyzed. The model included the treatment (Control or SB) and the batch as random effect. For sow and piglet blood and plasma data, time-related variations in concentrations were analyzed using the REPEATED statement. The model included the effects of treatment, sampling day, and their interaction. Differences between treatments were considered significant if $P < 0.05$. The PDIFF option of SAS adjusted to TUKEY comparisons test (for performance and milk data) or to BONFERRONI test (for blood repeated data) was used when significant differences were detected. Results are reported as adjusted least square means (**LSMEANS**) ± SEM.

Mortality rates were analyzed by the GENMOD procedure using a binomial error distribution and a logit-transformation, in a model that included the effects of treatment, parity, batch, and the interactions. The PDIFF option was used when a significant interaction was detected. Daily fecal scores were analyzed with the FREQ procedure to determine the daily prevalence of pens within each level of scores for each treatment (0, 1 and greater than 1).

Microbiota statistical analyses were carried out using R software (version 3.6.1). Vegan package was used to calculate OTUs Bray-Curtis dissimilarity matrix and dissimilarities in microbial composition were tested using multivariate ADONIS function. The beta-diversity, i.e., diversity between samples, was visualized using non Metric Dimensional Scaling (nMDS) ordination on OTUs Bray-Curtis dissimilarity matrix. A mixed linear model accounting for the fixed effect of the sampling day, the treatment and their interaction, as well as the random effect of the animal was applied to analyze the alpha-diversity. ANOVA with Satterthwaite correction of degree of freedom and Tukey's multiple comparisons of means were performed using lmerTest and emmeans R packages.

Differential analyses were applied on taxa detected at least in half of the samples of at least 1 group. A centered log-ratio transformation was applied to the relative abundance data as advised for compositional data (Aitchison, 1982). A mixed linear model accounting for the sampling day, the treatment and their interaction as fixed effects, and the animal as the random effect was applied. Normality of the model residues was assessed with a Shapiro test. Normality was considered acceptable when $P < 0.01$ and ANOVA was performed. Alternatively, non-parametric tests were applied to test the effects of sampling day and treatment (Prentice test) and the combination of sampling day and treatment (Kruskal-Wallis test). *P*-values were adjusted using Benjamin-Hochberg procedure.

Sparse Partial Least Square Discriminant Analysis (sPLS-DA), a supervised classification method, was applied for each sampling day, and for sows and piglets separately. That multivariate statistical method allows identifying the OTUs that contribute the most to discriminate the samples according to the treatment of the sows (MixOmics package, Rohart

et al., 2017). First, the OTU count table was normalized by total sum scaling after the addition of a pseudo count (0.001), filtered to keep only OTUs that represent at least 0.01% of the total sequences and subjected to centered log-ratio transformation. To assess whether the 2 treatment groups could be discriminated, PLS-DA was first performed. An iterative cross-validation (perf function with leave-one-out option) was used to assess the robustness of the discrimination. The calculated error-rate was used to validate the discrimination, an error-rate lower than 45% was considered acceptable. Sparse PLS-DA was then applied to select the most discriminant OTUs. The number of components, i.e., new variables created as linear combination of OTUs, and of OTUs to keep in the sPLS-DA model was optimized based on calculated error rate. A Wilcoxon Rank Sum test was finally used to test whether the relative abundances of the discriminant OTUs were significantly affected by the treatment when examined using a univariate approach.

## RESULTS

### *General observations*

Three sows were excluded from the experiment: 1 sow from the SB group aborted midway through gestation and 2 sows had a high number of stillborn piglets (1 in each experimental group). In total, 280 and 255 piglets born from Control and SB sows were included in the postweaning trial and were allotted in 27 pens of 10.4 piglets per pen on average for piglets born from Control sows and 26 pens of 9.8 piglets per pen for piglets born from SB sows.

347 During the 35 d of the postweaning period, three piglets from the SB group died because of
348 digestive disorders.
349
350 ***Sow Body Condition, Reproductive and Lactation Performance***
351 Sows of the 2 experimental groups had similar average parity (3.1 ± 0.3, $P > 0.10$). They had
352 similar BW and backfat thickness at the different physiological stages (226.7 ± 6.6 kg and
353 16.4 ± 0.4 mm, 272.1 ± 6.2 kg and 17.7 ± 0.4 mm, and 243.0 ± 6.4 kg and 14.2 ± 0.4 mm on
354 G28, G106, and L28 respectively). Their gain of body weight (45.4 ± 1.8 kg) and backfat
355 thickness (1.4 ± 0.3 mm) during gestation and loss of body weight (-11.5 ± 2.1 kg) and
356 backfat (-3.5 ± 0.2 mm) during lactation did not differ between the two groups of sows ($P >$
357 0.05). Treatment did not influence ($P > 0.10$) average daily feed intake (**ADFI**) of sows
358 during gestation (from G28 to the d of parturition: 2.80 ± 0.03 kg/d) and lactation (from L1 to
359 L28: 7.77 ± 0.17 kg/d). Litter sizes at birth, after cross-fostering (L2), on L6 and L28 did not
360 differ between treatments ($P > 0.10$, Table 1). The proportion of piglets born alive that died
361 before weaning was greater ($P < 0.05$) in litters born from SB sows than in those from Control
362 sows (Table 1). Nevertheless, part of the extra mortality in SB litters was due to a great
363 number of splayleg piglets in 2 of these litters (4 and 5 splayleg piglets, respectively). The
364 difference in mortality rates was no longer significant after exclusion of these 2 litters from
365 the analysis (Table 1).
366 Daily litter weight gain during lactation did not significantly differ between treatments
367 ($P > 0.05$, Table 1). Composition of colostrum and milk, in terms of nutrients, energy and
368 immunoglobulins was not significantly influenced by treatment ($P > 0.05$, Table 2). A

369 significant treatment x parity interaction was observed for the total amount of minerals (ash)
370 in milk, with ash concentrations being lower ($P < 0.05$) in milk from Control primiparous
371 sows than in milk from the 3 other groups of sows (0.70 ± 0.02%, 0.78 ± 0.01%, 0.78 ±
372 0.02% and 0.77 ± 0.01% in Control primiparous, Control multiparous, SB primiparous, and
373 SB multiparous sows, respectively).
374
375 ***Health and Metabolic Status of Sows***
376 No significant treatment x day interaction was observed for the criteria presented in Tables 2
377 and 3, except for lactate. Hematological variables of sow blood markedly fluctuated over time
378 ($P < 0.001$) without significant treatment effect ($P > 0.10$, Table 3). White blood cell count,
379 including lymphocytes and neutral granulocytes, were greater ($P < 0.05$) on G28 than on
380 G113 and L28. When expressed as percentages of white blood cells, the proportion of
381 lymphocytes decreased during gestation and further decreased during lactation. The count of
382 red blood cells and the blood concentration in hemoglobin also decreased during gestation and
383 then during lactation ($P < 0.05$, Table 3).
384 Haptoglobin showed greater concentrations in plasma at the end of gestation and lactation
385 than on G28 ($P < 0.05$) but no variation in response to treatment (Table 3). Plasma antioxidant
386 capacity (BAP) was lower ($P < 0.05$) on G113 than on L28 and was not influenced by
387 treatment ($P > 0.10$, Table 3). In contrast, dROM concentrations did not show significant
388 variation over time but they were greater in SB than in Control sows across sampling days
389 (1234 ± 24 vs 1108 ± 24 CarrU, $P < 0.05$, respectively). This difference including G28, before
390 the treatment began, it is not due to the supplementation with living yeasts.

391 Sow metabolic status was assessed by plasma concentrations of various metabolites and
392 cortisol in samples collected from fasted sows (Table 4). Independently of sampling day, SB
393 sows had greater concentrations of plasma urea (225.7 ± 5.7 vs 208.8 ± 5.8 mg/L, $P < 0.05$).
394 As for dROM, the difference included G28 and thus is not due to the supplementation with
395 living yeasts. Another treatment-independent difference between the 2 groups of sows was
396 observed for lactate concentrations on G28, with Control sows having lower concentrations
397 ($P < 0.05$). Neither glucose, FFA and creatinine, nor cortisol were influenced by treatment
398 ($P > 0.05$, Table 4). Unsurprisingly, variations over time were observed for glucose, FFA,
399 creatinine and urea. Glucose concentrations were lower at the end of lactation than during
400 gestation whereas concentrations of FFA and urea concentrations were greater ($P < 0.05$).
401 Creatinine concentrations were greater at the end of gestation than on G28 or L28 (Table 4).
402
403 ***Pig Performance and Fecal Scoring after Weaning***
404 On L28 and W35, the pen weight (Table 5) did not differ between the 2 treatments ($P > 0.05$).
405 Average daily feed intake (**ADFI**), average daily gain (**ADG**) and feed conversion ratio
406 (**FCR**) calculated at the pen level between L28 and W35 did not differ ($P > 0.05$). The
407 percentage of pens observed with a score value of 0, 1 or greater than 1 throughout the post-
408 weaning period did not differ between treatments (Khi-2 test, $P > 0.95$).
409
410 ***Pig Blood Variables Before and After Weaning***
411 Neither the effect of treatment nor the interaction between treatment and sampling day were
412 significant ($P > 0.09$) on any blood variables. Weaning induced dramatic changes in nearly all

the variables measured in our study (Table 6). It induced changes in the white blood cell population ($P < 0.001$) with greater counts of total white blood cells, lymphocytes and neutral granulocytes on W5 ($P < 0.001$, Table 6) than on L28. On W5, the proportion of lymphocytes was lower whereas that of neutral granulocytes was greater than on L28 ($P < 0.001$). The count of red blood cells was greater while hemoglobin concentrations were lower ($P < 0.001$) on W5 than L28. Piglets born from Control sows had lower hemoglobin concentrations irrespective of the day of blood sampling ($P = 0.04$) and hemoglobin concentrations were lower after than before weaning ($P = 0.04$). Haptoglobin and dROM plasma concentrations were greater on W5 irrespective of the treatment ($P < 0.001$, Table 6) whereas BAP did not differ between L28 and W5. The treatment had no effect on these 3 variables before and after weaning.

***Sow and Piglet Fecal Microbiota***

A total of 164 samples from sows (21 control and 20 SB, 4 timepoints) and 165 samples from piglets (between 24 and 30 per treatment and timepoint group) were analyzed. After quality filtering and chimera removal, 28478 ± 6143 reads were kept per sample. After rarefaction, OTUs represented by less than 10 reads were discardered, an abundance table containing 5860 OTUs was generated and taxonomic binning was performed. As expected, a strong effect of the day of sampling on the fecal microbiota composition was evidenced from the beta-diversity for sows and piglets (Supplementary Fig.1). In sows, the relative abundances of *Firmicutes* and *Actinobacteria* phyla were lower whereas those of *Bacteroides* were higher during lactation than during gestation. The abundance of *Spirochaetes* decreased between

G110 and L6 (supplementary Table 1, phylum table). The relative abundance of the *Epsilonbacteraeota*, mainly represented by bacteria belonging to *Campylobacteraceae* family, was lower on L6 in both groups. Interestingly, on L28, it did not differ anymore from the relative abundance measured on G110 in the Control sows whereas it was still lower in the SB sows. Compared to gestation, *Proteobacteria* relative abundance was greater on L6 and L28 in the SB sows and only on L28 for the Control sows. Among the 145 genera tested (supplementary Table 1, genus table), 84 were significantly affected by the physiological status of the sows ($P < 0.05$). Major changes in relative abundance (absolute value of log2 fold change > 2) were observed for *Mitsuokella*, *Lachnoclostridium 10* and *Olsenella*, which increased between G28 and G110 and decreased after farrowing. *Blautia*, *Sarcina*, *Coprococcus* 3, *Faecalibacterium*, *Lachnospiraceae UCG-007* and *Anaerostipes* were more abundant during lactation than during gestation (supplementary Table 1, genus table). As expected, major modulation of the feces microbiota occurred as the piglets aged. The relative abundances of almost all tested taxa (phylum, family and genus levels) were shifted between L6 and L28 (Supplemental table 2). In sows, richness of fecal microbiota (Table 7) was not affected by the day of sampling ($P = 0.68$) while Shannon diversity index slightly decreased between G28 and G110 ($P < 0.05$) and did not differ between L6 and L28 ($P > 0.05$). The richness was lower ($P < 0.05$) on L28 in SB sow compared with Control sows (Table 7). In piglets (Table 8), as expected, fecal microbiota richness and Shannon index increased between L6 and L28. Five days after weaning, richness and shannon index were lower ($P < 0.05$) than before weaning in the SB group only.

To assess the effect of SB supplementation on fecal microbiota composition at OTU level, a PLS-DA analysis was carried out separatly on sow and piglet data, for each day of sampling. This multivariate approach allowed to consider the combined effect of all OTUs. For sows, a validated PLS-DA model allowed to discriminate between SB and Control groups on G110 and L28, whereas the model was not validated on G28 and L6 (error rate > 45%, Table 9). Interestingly, piglet samples could be discriminated according to the group of their mother for all days of sampling, including after weaning. Nevertheless, the performance of the PLS-DA was better on L6 than on W5 (3.8% vs 17% error rate). The discrimination between treatment groups was notaceably more robust when considering the piglets than when considering the sows (3.8% *vs* 49% and 7% *vs* 36.7% error rate, respectively for piglets and sows on L6 and L28, Table 9). The most discrimininative OTUs were then identifed using sPLS-DA. Depending on the day of sampling, 39 to 61 OTUs were selected to optimize the performance of the sPLS-DA model (Table 9). The median relative abundance of the 15 most discriminative OTUs is presented in Figures 1 and 2. On G110, compared to the microbiota of the Control sows, the fecal microbiota of the SB sows were mainly characterized with higher abundance of OTUs belonging to *Ruminococcus*, *Coprostanoligenes* group, *Prevotellaceae NK3B31* group, *Subdoligranulum*, *Blautia*, *Lachnoclostridium* and *Marvinbryantia*. Discriminant OTUs belonging to *Ruminococcaceae UGC014* and *NK4A214* groups, *Cellulolyticum* and *Fusobacterium* were otherwise more abundant in control sows. At weaning, the most discriminative OTUs for the microbiota of the SB sows belong to *Roseburia*, *Bacteroides*, *Alloprevotella* genera and *Bradymonadales* family, whereas OTUs

affiliated to *Campylobacter* and *Prevotellaceae UCG004* group were less abundant in control sows.

In piglets, amongst the 15 most discriminant OTU, relative abundances of OTUs belonging to *Lachnoclostridium* (2 OTUs), *Christensenellaceae R7* group, *Lactobacillus* (2 OTUs), *Helococcus* and *Bacteroides* genera were higher on L6 in feces of piglets born from SB sows compared with Control group. Conversely, OTUs belonging to *Erysipelotrichaceae* family (2 OTUs) and to *Fusobacterium* (2 OTUs), *Tyzzerella* and *Coprococcus* genera were less abundant in SB piglets when compared to Control piglets. On L28, the fecal microbiota of the piglets from SB group could be discriminated from the control group with higher abundances of OTUs affiliated to *Catenisphaera*, *Rikenellaceae RC9* group, *Blautia*, *Solobacterium* and lower abundances of *Ruminococcaceae* (3 OTUs), *Bacteroides*, *Flavonifractor* and *Peptococcus*. After weaning and compared to piglets born from Control sows, out of the 15 most discriminative OTUs, OTUs belonging to *Mitsuokella* genus (4 OTUs) and *Ruminococcus 1* (2 OTUs) genera were less abundant, while OTUs annotated as *Alloprevotella*, *Lactobacillus* (2 different OTUs from those selected on L6) and *Faecalibacterium* were more abundant in the piglets born from SB sows.

## DISCUSSION

Our study showed that SB supplementation in sow diet during gestation and lactation induced modifications in the fecal microbiota of sows and their piglets during lactation and after

498 weaning. These modifications were however associated with changes neither in piglet ability
499 to cope with the stress of weaning, nor in milk nutritional and immune composition.
500 To assess the potential benefit of SB supplementation in the maternal feed on robustness of
501 piglets at weaning, piglets were weaned in non-optimal conditions. This consisted in
502 transferring pigs in uncleaned pens, at a non-optimal temperature for a short period of time
503 and mixing pigs from different litters. Such non-optimal housing conditions have been tested
504 to induce a systemic inflammatory response and an oxidative stress at weaning (Buchet et al.,
505 2017) and during the growing period (Chatelet et al., 2018). Accordingly, in the present
506 experiment, weaning successfully induced a systemic inflammation in piglets confirmed by
507 greater white blood cell count and plasma concentrations in haptoglobin and dROM. Piglets
508 born from sows fed SB during both gestation and lactation did not grow faster before and
509 after weaning. Our results did not confirm previous findings (Tan et al., 2015) showing that
510 piglets born from sows fed the same dose of SB during 2 consecutive reproductive cycles
511 were heavier at weaning. In that study, the authors did not report any positive effect on piglets
512 born after the first gestation suggesting that a longer period of distribution might be necessary
513 to induce effects that sows could transfer to their litter. To our knowledge, our study is the
514 first one that investigates the effect of SB supplemented in sow diet on postweaning pigs.
515 However, after weaning either, SB supplementation in the maternal feed did not improve the
516 piglet capacity to cope with the stressful conditions of weaning when considering the
517 prevalence and severity of diarrhea, and blood indicators of inflammation and oxidative
518 status, that did not differ in piglets born from SB and Control sows.

519 Blood concentration in hemoglobin is an indicator of iron status and a key parameter to
520 evaluate iron deficiency anemia in young piglets (Szudzik et al., 2018). Weaning induced a
521 slight decrease in hemoglobin concentrations but these concentrations remained greater than 9
522 g/dL, the threshold value for anemia and considered as a level at which optimal performance
523 may occur (Knight and Dilger, 2018). However, piglets born form SB sows had greater
524 hemoglobin blood concentrations than Control piglets. In young pigs, an increase in
525 *Lactobacillus* and *Bifidobacterium* populations caused by inulin supplementation was
526 associated with increased expression of genes coding for iron transporters in the intestine and
527 blood hemoglobin concentration (Tako et al., 2008). Interestingly, our study showed that
528 some *Lactobacillus* OTUs were more abundant in piglets born from SB sows, on L6 and W5.
529 The effects of SB on microbiota composition, specifically on *Lactobacillus* species, and iron
530 absorption and status would deserve attention.
531 Regarding the maternal side, the SB supplementation also had no effect on performance
532 and physiological traits. Parameters measured to estimate sow body condition and metabolic
533 status did not differ in response to SB supplementation, neither at the end of gestation nor at
534 the end of lactation. In our study, sow feed intake during lactation also was not influenced by
535 SB supplementation. Our results contrasted with those from Sun et al. (2021) who reported
536 greater feed intake during the first week of lactation in sows fed SB from the late gestation.
537 Similarly, in tropical humid climate, sows fed the same SB strain as in the present study, from
538 late gestation and throughout lactation, presented a greater feed intake during lactation and a
539 trend for less fat tissue mobilization (Domingos et al., 2021). The impact of SB
540 supplementation might therefore depend on the environmental conditions of the sows. In

addition, the ADFI of the Control sows during the lactation in our study was higher than the ADFI reported in the other studies, suggesting that the Control sows already expressed their full intake potential. As with metabolic status, the health level of sows did not appear to be affected, since markers of the inflammatory, oxidative and immune status did not respond to SB supplementation. The metabolic status and health level of the sows being not affected, it is not really surprising that colostrum and milk composition was not affected either. The only difference was the greater concentrations of ash, therefore of minerals, in milk of primiparous sows that received SB than in milk from Control primiparous sows. Because of the low number of primiparous sows in the experiment (7/treatment), this effect needs to be substantiated before any interpretation. More surprising, however, was the lack of impact of SB supplementation on immunoglobulin concentration in colostrum or milk. A supplementation of the same strain of SB during the last 3 weeks of gestation significantly increased colostral concentration of IgG by 21% and those of IgA by 18% (Guillou et al., 2012). The dose of SB provided to sows was much greater in their study than in the present one ($5x10^{10}$ CFU/d vs between 2.5 and $3.2x10^{9}$ CFU/d). Supplementation with other *Saccharomyces cerevisiae* strains were also shown to increase IgG concentrations in colostrum (Zanello et al., 2013) or in piglet plasma 24 h after birth (Jang et al., 2013). In the present experiment, piglet and litter performance during lactation was assessed through survival and growth rate. Supplementation of sow diet with SB affected neither rates of mortality between cross-fostering and weaning, nor piglet and litter growth rate, which reflected no effect on milk production. In tropical humid climate, SB supplementation during late gestation and lactation increased milk production by 9% (Domingos et al., 2021). In

563 temperate climate, however, both positive and no effect was observed on piglet or litter
564 growth rate (Di Giancamillo et al., 2007; Bravo de Laguna et al., 2020). These results would
565 suggest that SB would exert positive effects on sows in non-optimal conditions. Overall, the
566 performance and health of sows and piglets included in our study were good and may have
567 hidden any improvement of these phenotypes by SB.
568 The gut microbiota composition in sows is affected by various environmental factors
569 including physiological stage and parity, diet fiber content or environmental stress (Leblois et
570 al., 2018; Liu et al., 2019a; Gaukroger et al., 2021; Lührmann et al., 2021). In accordance
571 with these studies, we observed over-time variations in the gut microbiota of sows during
572 gestation and lactation. In our study, SB supplementation slightly altered fecal microbiota
573 composition of the sows at the end of the gestation (G110) and at the end of lactation (L28).
574 Energy requirement for fetus growth at the end of gestation and for milk production is high
575 during these periods. The interaction between host (sow) and its microbiota might be altered
576 by this high physiological demand, which could lead to microbiota permissiveness for SB
577 action. Supplementation with SB mostly modified the balance of well-known fiber degrader
578 commensal bacteria (i.e. *Ruminococcus*, *Lachnospiraceae*, *Blautia*, *Cellulosyliticum*,
579 *Bacteroides*…). Species belonging to beneficial bacteria such as *Subdoligranulum* and
580 *Christensenellaceae R7* group were more abundant in SB sows, while potential pathogens
581 such as *Fusobacterium* and *Campylobacter* were found in higher abundance in Control sows.
582 Strikingly, despite no effect on milk immune and nutritional composition, and only slight
583 effects on sow feces microbiota composition, sow diet supplementation with SB elicited
584 strong effects on the piglet gut microbiota at every age of feces sampling as evidenced by the

585 discriminant analyses error rates. This may be explained by the high plasticity of piglet
586 microbiota compared to adults (Derrien et al., 2019). Indeed, during lactation, the piglet gut
587 microbiota is colonized by bacteria from its environment and the sow feces (Liu et al.,
588 2019b). Its composition is also influenced by the composition of the milk from the nursing
589 mother (Bian et al., 2016). To our knowledge, the effect of supplementation of sow diets with
590 SB during gestation and lactation on offspring gut microbiota had never been reported before.
591 For instance, the *Lactobacillus* genus was more abundant in SB piglets on W5. Members of
592 *Lactobacillus* genus are known as favorable for host. Indeed, *L. frumenti* and *gasseri* have
593 been associated with lower incidence of diarrhea in weaned piglets (Hu et al., 2018).
594 Moreover, the lowest enrichment or absence of members of *Clostridium innocuum*,
595 *Fusobacterium* and *Tyzzerella* genera on L6 in piglets born from SB sows are interesting
596 since members of these genera have high proteolytic activity and may be responsible for
597 piglet neonatal diarrhea (Hermann-Bank et al., 2015; Chia et al., 2017). The effect of SB
598 supplementation on piglet microbiota was not only maintained during lactation, but also 5 d
599 after weaning. This shows the persistence of the effect of the sow diet supplementation on
600 piglet microbiota, although SB supplementation in sow diet had a lower impact around
601 weaning than during the first week of lactation. In weaned piglets, SB signature on fecal
602 microbiota included an enrichment of members of the *Lactobacillus* and *Faecalibacterium*
603 genus associated with low abundance of several *Mitsuokella* members when compared to
604 Control group. Of note the *Lactobacillus* OTUs enriched 5 d after weaning were not the same
605 than the ones enriched in piglets on L6. This result is consistent with previous observation
606 (Wang et al., 2019) that described a *Lactobacillus* abundant after weaning but that was

607 undetectable during suckling period. *Faecalibacterium* has been associated with late weaning
608 and has potentially beneficial effect on health and growth (Massacci et al., 2020).
609 In conclusion, dietary supplementation of SB to sows did not elicit any changes on piglet
610 performance and health before and after being challenged at weaning. It did change neither
611 sow's reproductive performance, metabolic and health status, nor in the immunoglobulin and
612 nutrient content of colostrum and milk. In our experimental conditions, feeding SB to sows
613 favored the development of beneficial microbes in sows and piglets. Further studies would be
614 necessary to examine if and how these beneficial microbes would confer an advantage to the
615 piglets. Moreover the transmission of the sow microbiota to the piglets and how it could be
616 modulated by the feed and probiotic supplementation would deserve a specific attention.
617
618 **Conflict of Interest:** The authors declare no conflict of interest.
619
620 **LITERATURE CITED**
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782

783 **Table 1.** Performance of litters born from sows fed no dietary supplementation (Control) or
784 living yeasts (SB) from d 28 of gestation until d 28 of lactation.[1]

| | Treatment | | | *P*-value | | |
|---|---|---|---|---|---|---|
| Item | Control | SB | SEM | T | Parity | T x P[2] |
| No. of litters | 23 | 22 | | | | |
| Number of piglets/litter | | | | | | |
| Born (total) | 16.1 | 16.2 | 0.8 | 0.92 | 0.23 | 0.65 |
| Born alive | 15.5 | 15.7 | 0.7 | 0.80 | 0.76 | 0.98 |
| After cross-fostering | 15.5 | 15.9 | 0.6 | 0.66 | 0.73 | 0.75 |
| At weaning | 12.5 | 11.6 | 0.7 | 0.15 | 0.23 | 0.22 |
| Litter weight, kg | | | | | | |
| At birth (all piglets) | 21.5 | 21.8 | 1.1 | 0.81 | <0.001 | 0.92 |
| After cross-fostering | 21.6 | 22.0 | 1.0 | 0.74 | 0.001 | 0.93 |
| At weaning | 105.9 | 97.9 | 5.1 | 0.08 | 0.01 | 0.18 |
| Litter weight gain during lactation, kg/d | 2.93 | 2.65 | 0.16 | 0.10 | 0.11 | 0.24 |
| Mortality rates, % | | | | | | |
| At birth | 5.3 | 3.5 | 1.4 | 0.20 | <0.001 | 0.05 |
| Cross fostering-weaning | 20.2 | 25.6 | 3.0 | 0.03 | 0.17 | 0.06 |
| Cross fostering-weaning[3] | 20.2 | 24.0 | 3.0 | 0.15 | 0.06 | 0.24 |

785 [1] Data are expressed as least-squares means and the greatest SEM, except for mortality rates
786 (raw data).

[2] T x P: treatment x parity (primiparous vs multiparous) interaction.

[3] Mortality rates after excluding 2 SB litters with 4 and 5 splayleg piglets.

799 **Table 2.** Colostrum and milk composition in sows fed no dietary supplementation (Control)

800 or living yeasts (SB) from d 28 of gestation until d 28 of lactation.[1]

| Item | Treatment | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|
| | Control | SB | | T | Parity | T x P[2] |
| Colostrum | | | | | | |
| Dry matter[3], % | 27.5 | 27.4 | 1.0 | 0.92 | 0.19 | 0.30 |
| Ash[3], % | 0.63 | 0.65 | 0.01 | 0.39 | 0.85 | 0.94 |
| Protein[3],% | 16.32 | 16.67 | 0.55 | 0.61 | 0.44 | 0.63 |
| Fat[3], % | 5.29 | 5.09 | 0.49 | 0.78 | 0.34 | 0.36 |
| Lactose[3], % | 2.73 | 2.54 | 0.07 | 0.06 | 0.03 | 0.37 |
| Gross energy, kJ/g | 6.78 | 6.80 | 0.30 | 0.95 | 0.08 | 0.24 |
| IgG, mg/mL | 63.33 | 64.57 | 5.40 | 0.87 | 0.28 | 0.92 |
| IgA, mg/mL | 12.05 | 11.10 | 1.97 | 0.48 | 0.06 | 0.77 |
| Milk on d 6 of lactation | | | | | | |
| Dry matter[3], % | 19.00 | 18.87 | 0.34 | 0.77 | 0.40 | 0.53 |
| Ash[3], % | 0.74 | 0.78 | 0.01 | 0.05 | 0.03 | 0.01 |
| Protein[3],% | 5.24 | 5.29 | 0.11 | 0.69 | 0.002 | 0.54 |
| Fat[3], % | 7.40 | 7.11 | 0.29 | 0.49 | 0.05 | 0.51 |
| Lactose[3], % | 5.15 | 5.10 | 0.09 | 0.53 | 0.87 | 0.91 |
| Gross energy, | 5.02 | 4.82 | 0.11 | 0.22 | 0.37 | 0.87 |

| | | | | | | |
|---|---|---|---|---|---|---|
| kJ/g | | | | | | |
| IgA, mg/mL | 1.28 | 1.65 | 0.20 | 0.18 | 0.04 | 0.89 |

[1] Data are expressed as least-squares means, and the greatest SEM.

[2] T x P: treatment x parity (primiparous vs multiparous) interaction.

[3] Grams per 100 g of whole colostrum or milk.

805 **Table 3.** Blood hematological variables and plasma markers of inflammation and oxidative
806 stress in sows fed no dietary supplementation (Control) or living yeasts (SB) from d 28 of
807 gestation until d 28 of lactation.[1]

| | Treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | | | SB | | | | *P*-value[2] | |
| Item[3] | G28 | G113 | L28 | G28 | G113 | L28 | SEM | T | Day |
| White blood cells, 1,000/µL | 13.4$^{A}$ | 10.7$^{B}$ | 11.1$^{C}$ | 13.9$^{A}$ | 10.7$^{B}$ | 12.3$^{C}$ | 0.50 | 0.33 | < 0.001 |
| Lymphocytes, 1,000/µL | 7.6$^{A}$ | 5.0$^{B}$ | 4.0$^{C}$ | 7.7$^{A}$ | 4.7$^{B}$ | 4.4$^{C}$ | 0.25 | 0.76 | < 0.001 |
| Granulocytes, 1,000/µL | 5.1$^{A}$ | 4.8$^{A}$ | 6.1$^{B}$ | 5.5$^{A}$ | 5.0$^{A}$ | 6.4$^{B}$ | 0.34 | 0.27 | < 0.001 |
| Lymphocytes, % | 57.0$^{A}$ | 47.1$^{B}$ | 35.5$^{C}$ | 55.6$^{A}$ | 44.0$^{B}$ | 35.6$^{C}$ | 1.55 | 0.20 | < 0.001 |
| Granulocytes, % | 38.1$^{A}$ | 44.0$^{B}$ | 52.6$^{C}$ | 39.9$^{A}$ | 46.8$^{B}$ | 52.5$^{C}$ | 1.55 | 0.24 | < 0.001 |
| Red blood cells, 1,000000/µL | 6.6$^{A}$ | 5.8$^{B}$ | 5.2$^{C}$ | 6.8$^{A}$ | 5.6$^{B}$ | 5.1$^{C}$ | 0.14 | 0.75 | < 0.001 |
| Hemoglobin, g/dL | 13.6$^{A}$ | 11.4$^{B}$ | 10.7$^{C}$ | 13.6$^{A}$ | 11.2$^{B}$ | 10.1$^{C}$ | 0.20 | 0.09 | < 0.001 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Haptoglobin, mg/mL | $0.91^{A}$ | $1.76^{B}$ | $1.97^{B}$ | $0.75^{A}$ | $1.73^{B}$ | $1.99^{B}$ | 0.13 | 0.58 | < 0.001 |
| BAP, µM Eq vitamin C | $2529^{AB}$ | $2458^{A}$ | $2529^{B}$ | $2461^{AB}$ | $2443^{A}$ | $2563^{B}$ | 23 | 0.37 | < 0.001 |
| dROM, CarrU | 1151 | 1141 | 1033 | 1253 | 1247 | 1202 | 43 | < 0.001 | 0.08 |

[1] Data are expressed as least-squares means, and the greatest SEM.

[2] T: Treatment effect; Day: sampling day effect. Irrespective of the treatment group, values with different superscripts A, B, C differed ($P < 0.05$, sampling day effect).

[3]BAP = Biological Antioxidant Power; CarrU = "Carratelli Units", where 1 CARRU is equivalent to the oxidizing power of 0.08 mg $H_2O_2$/dL.

**Table 4.** Concentrations of metabolites and cortisol in plasma of sows fed no dietary supplementation (Control) or living yeasts (SB) from d 28 of gestation until d 28 of lactation.[1]

| | Treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | | | SB | | | | *P*-value[3] | |
| Item[2] | G28 | G113 | L28 | G28 | G113 | L28 | SEM | T | Day |
| Glucose, mg/L | 734.2$^{A}$ | 755.4$^{A}$ | 657.3$^{B}$ | 738.1$^{A}$ | 738.2$^{A}$ | 609.1$^{B}$ | 20.3 | 0.17 | < 0.001 |
| Lactate[4], mM | 1.8$^{a}$ | 2.3$^{b}$ | 2.4$^{b}$ | 2.3$^{b}$ | 2.0$^{b}$ | 2.2$^{b}$ | 0.1 | 0.88 | 0.26 |
| FFA, µM | 123.2$^{A}$ | 202.9$^{A}$ | 886.1$^{B}$ | 97.0$^{A}$ | 271.0$^{A}$ | 1155.0$^{B}$ | 72.5 | 0.06 | < 0.001 |
| Creatinine, mg/L | 17.5$^{A}$ | 23.7$^{B}$ | 18.0$^{A}$ | 17.8$^{A}$ | 24.2$^{B}$ | 17.7$^{A}$ | 0.5 | 0.61 | < 0.001 |
| Urea, mg/L | 162.7$^{A}$ | 176.6$^{A}$ | 287.1$^{B}$ | 178.1$^{A}$ | 181.4$^{A}$ | 317.6$^{B}$ | 10.2 | 0.04 | < 0.001 |
| Cortisol, mg/L | 44.3 | 46.6 | 53.1 | 39.3 | 48.6 | 53.2 | 7.8 | 0.86 | 0.06 |

[1] Data are expressed as least-squares means, and the greatest SEM.

[2] Free fatty acids.

[3]T: Treatment effect; Day: sampling day effect. Irrespective of the treatment group, values with different superscripts A, B, C differed ($P < 0.05$, sampling day effect).

[4]Treatment x Day interaction: $P = 0.02$ (values with different superscripts a, b differed ($P < 0.05$).

826 **Table 5.** Postweaning average performance per pen of piglets born from sows fed no dietary
827 supplementation (Control) or living yeasts (SB) from d 28 of gestation until d 28 of lactation.[1]

| | Treatment | | | |
|---|---|---|---|---|
| Item[2] | Control | SB | SEM | *P*-value[3] |
| Weaning | | | | |
| Pen average weight, kg | 91.3 | 85.5 | 3.20 | 0.21 |
| Piglet average weight, kg | 8.84 | 8.77 | 0.34 | 0.89 |
| 35 d after weaning | | | | |
| Pen average weight, kg | 245.0 | 226.5 | 11.0 | 0.08 |
| Piglet average weight, kg | 23.7 | 23.2 | 1.2 | 0.64 |
| Pen ADG, kg/(d.piglet) | 0.436 | 0.424 | 0.02 | 0.57 |
| Pen FI, kg | 214.9 | 200.0 | 6.40 | 0.11 |
| Pen ADFI, kg/(d.piglet) | 0.743 | 0.734 | 0.04 | 0.81 |
| pen FCR, kg/(d.piglet) | 1.38 | 1.41 | 0.04 | 0.37 |

828 [1] Data are expressed as least-squares means, and the greatest SEM. The experimental unit is
829 the pen.
830 [2]ADG: average daily gain; FI: feed intake; ADFI: average daily feed intake; FCR: feed
831 conversion ratio.
832 [3]Treatment effect.
833

834 **Table 6.** Blood hematological variables and plasma markers of inflammation and oxidative
835 stress at weaning and 5 days after weaning in piglets born from sows fed no dietary
836 supplementation (Control) or living yeasts (SB) from d 28 of gestation until d 28 of lactation.[1]

| Item[3] | Treatment | | | | SEM | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | SB | | | | | |
| | L28 n = 45 | W5 n = 44 | L28 n = 43 | W5 n = 43 | | T | Day | T x Day |
| White blood cells, 1,000/µL | 10.5 | 12.8 | 10.1 | 12.9 | 0.61 | 0.84 | <0.001 | 0.50 |
| Lymphocytes, 1,000/µL | 6.59 | 7.62 | 6.25 | 7.20 | 0.26 | 0.24 | <0.001 | 0.86 |
| Lymphocytes, % | 65.2 | 63.2 | 65.6 | 60.3 | 2.02 | 0.42 | <0.001 | 0.09 |
| Granulocytes, 1,000/µL | 2.29 | 3.29 | 2.36 | 3.73 | 0.36 | 0.33 | <0.001 | 0.29 |
| Granulocytes, % | 23.7 | 26.1 | 23.2 | 28.6 | 1.85 | 0.46 | <0.001 | 0.12 |
| Red blood cells, 1,000000/µL | 6.49 | 6.62 | 6.57 | 6.81 | 0.09 | 0.07 | <0.001 | 0.22 |
| Hemoglobin, g/dL | 10.3 | 9.8 | 10.5 | 10.3 | 0.25 | 0.04 | 0.04 | 0.52 |
| Haptoglobin, g/L | 0.15 | 1.95 | 0.15 | 2.01 | 0.099 | 0.75 | <0.001 | 0.76 |
| BAP, µM Eq vitamin C | 2585 | 2562 | 2593 | 2600 | 39.7 | 0.39 | 0.74 | 0.51 |
| dROM, CARRU | 709 | 1089 | 732 | 1144 | 27.9 | 0.24 | <0.001 | 0.44 |

837 [1] Data are expressed as least-squares means, and the greatest SEM.

838 [2] T: Treatment effect; Day: sampling day effect; T x Day: Treatment x Day interaction.

[3]BAP = Biological Antioxidant Power; CarrU = "Carratelli Units", where 1 CARRU is equivalent to the oxidizing power of 0.08 mg $H_2O_2$/dL.

**Table 7.** Alpha-diversity in fecal microbiota of sows fed no dietary supplementation (Control) or living yeasts (SB) from d 28 of gestation until d 28 of lactation.[1]

| | Control (n = 21) | | | | SB (n = 20) | | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G28 | G110 | L6 | L28 | G28 | G110 | L6 | L28 | SEM | T | Day | T x Day |
| richness | 832$^{a}$ | 808$^{a}$ | 804$^{a}$ | 862$^{a}$ | 833$^{a}$ | 833$^{a}$ | 818$^{a}$ | 791$^{b}$ | 20.7 | 0.68 | 0.68 | 0.04 |
| Shannon index | 4.70$^{A}$ | 4.43$^{B}$ | 4.40$^{B}$ | 4.47$^{B}$ | 4.74$^{A}$ | 4.41$^{B}$ | 4.52$^{B}$ | 4.39$^{B}$ | 0.09 | 0.82 | <0.001 | 0.54 |

[1] Data are expressed as least-squares means, and the greatest SEM.

[2] T: Treatment effect; Day: sampling day effect; T x Day: Treatment x Day interaction.

Lowercase superscripts: Treatment effect tested by day, values with different superscripts are different ($P < 0.05$, Tukey adjustment).

Uppercase superscripts: overall sampling day effect, values with different superscripts are different ($P < 0.05$, Tukey adjustment, comparison of 3 estimates).

853 **Table 8.** Alpha-diversity in fecal microbiota of piglets from sows fed no dietary

854 supplementation (Control) or living yeasts (SB) from d 28 of gestation until d 28 of lactation.[1]

| | Control | | | SB | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | L6 | L28 | W5 | L6 | L28 | W5 | SEM | T | Day | T x Day |
| N | 29 | 30 | 30 | 24 | 27 | 25 | | | | |
| richness | 289[a] | 695[b] | 683[b] | 320[a] | 761[b] | 562[c] | 25.7 | 0.68 | <0.001 | <0.001 |
| Shannon index | 3.57[a] | 4.66[b] | 4.57[b] | 3.65[a] | 4.63[b] | 4.09[c] | 0.09 | 0.03 | <0.001 | 0.001 |

855 [1] Data are expressed as least-squares means, and the greatest SEM.

856 [2] T: Treatment effect; Day: sampling day effect; T x Day: Treatment x Day interaction.

857 Values with different superscripts are different ($P < 0.05$, Tukey adjustment).

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861

**Table 9.** Evaluation of the performance of the models to discriminate the fecal microbiota of sows fed no dietary supplementation (Control) or living yeasts (SB) from d 28 of gestation until d 28 of lactation and their piglets.

| | n | | OTU > 0.01%[1] | model performance[2] | | OTU selected[3] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | SB | | PLS-DA | sPLS-DA | comp 1 | comp 2 | comp 3 | total |
| Sows | | | | | | | | | |
| G28 | 21 | 20 | 675 | *56.7%* | - | - | - | - | - |
| G110 | 21 | 20 | 612 | 31.9% | 36.7% | 20 | 30 | - | 49 |
| L6 | 21 | 20 | 588 | *49.0%* | - | - | - | - | - |
| L28 | 21 | 20 | 631 | 36.7% | 29.4% | 20 | 15 | 10 | 44 |
| Piglets | | | | | | | | | |
| L6 | 29 | 24 | 361 | 3.8% | 3.4% | 40 | 10 | - | 39 |
| L28 | 30 | 27 | 649 | 7.0% | 8.9% | 45 | 10 | 10 | 61 |
| W5 | 30 | 25 | 605 | 13.0% | 12.7% | 20 | 5 | 25 | 48 |

[1] For each time point, only OTUs represented by more than 0.01% of the total sequences were kept.

[2]Best performance for up to 3 tested components; PLS-DA: Partial Least Square Discriminant Analysis; sPLS-DA: Sparse Partial Least Square Discriminant Analysis.

[3]Number of selected OTUs for each component (Comp); Component corresponds to a new variable created as linear combination of OTUs.

873 **Figure captions:**

874 **Figure 1.** Selection of most discriminative OTUs to discriminate the fecal microbiota of sows
875 fed no dietary supplementation (Control) or living yeasts (SB) from d 28 of gestation until d
876 28 of lactation.

877 The median relative abundances of the 15 most discriminant OTUs in the fecal microbiota of
878 sows before parturition at 110 d of gestation (G110, a) and at 28 d of lactation (L28, b) are
879 shown. The selected OTUs are ranked according to their importance in the sPLS-DA model
880 (absolute values of the loading values). Taxonomic annotations are given at the family, the
881 genus or the species level when relevant. Star(s) indicate a significant difference according to
882 a Wilcoxon Rank Sumtest (* $P< 0.05$; ** $P<0.01$).

883

884 **Figure 2.** Selection of most discriminative OTUs of fecal microbiota of piglets born from
885 sows fed no dietary supplementation (Control) or living yeasts (SB) from d 28 of gestation
886 until d 28 of lactation.

887 The median relative abundances of the 15 most discriminant OTUs in the fecal microbiota of
888 piglets at 6 d of lactation (L6, a), 28 d of lactation (L28, b) and 5 d after weaning (W5, c) are
889 shown. The selected OTUs are ranked according to their importance in the sPLS-DA model
890 (absolute values of the loading values). Taxonomic annotations are given at the family, the
891 genus or the species level when relevant. Star(s) indicate a significant difference according to
892 a Wilcoxon Rank Sum test (* $P< 0.05$; ** $P<0.01$).

893

894

**Supplementary figure 1.** Beta-diversity of the fecal microbiota of sows fed no dietary supplementation (Control) or living yeasts (SB) from d 28 of gestation until d 28 of lactation and their piglets evaluated by nMDS ordination using Bray-curtis dissimilarities discriminate. Sow samples were collected at 28 (G28) and 110 d (G110) of gestation and 6 (L6) and 28 d (L28) of lactation; piglet samples were collected at 6 (L6) and 28 d (L28) of lactation and 5 d after weaning (W5).