Standardized Natural Citrus Extract dietary supplementation influences sows' microbiota, welfare, and preweaning piglets' performances in commercial rearing conditions

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ABSTRACT: We investigated the effect of the Standardized Natural Citrus Extract (SNCE; Nor-Spice AB, Nor-Feed SAS, France) on the microbiota of the sows and on the weight gain of their piglets. Fifty sows were randomly divided into two groups: a control group (23 sows) with a standard diet and a SNCE group (27 sows) with a standard diet supplemented with 2,500 ppm of SNCE. Supplementation occurred 10 d before and 5 d after farrowing. Fecal samples from 16 sows (8 randomly selected sows of each dietary treatment) were collected for the fecal microbiota analysis 5 d after farrowing. The supplementation of SNCE increases the amount of cultivable *Lactobacillus* threefold in vitro. Microbial DNA was extracted from the fecal samples for sequencing of the 16S rRNA gene. The SNCE, which affected the microbiota as a discriminant analysis, was able to separate the microbial communities of the eight sows that received SNCE from the three control sows with 21 Operational Taxonomic Units (area under the ROC curve = 96%). SNCE also reduced the interval between farrowing and the first dejection of the sow and increased their feed intake (P-value < 0.05). Furthermore, feeding the sows with SNCE improved the weight gain of the piglets in the first week of life. These results show that SNCE supplementation allows to enhance zootechnical performances of peripartum' sows, possibly due to the modulation of the microbiota transmitted to the piglets.

Key words: *peripartum* sows, piglets, citrus extract, microbiota, zootechnical performances

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INTRODUCTION

Maintaining healthy animals is a key part of livestock breeding. In pig production, digestive disorders are common, particularly in critical periods such as farrowing (Tabeling et al., 2003). These digestive disorders are closely linked to dysbiosis of the gut microbiota (Pearodwong et al., 2016; Peltoniemi et al., 2016; Zhao and Yu, 2016) and can have dramatic consequences on the zootechnical performances and welfare of the pigs (Pearodwong et al., 2016; Cao et al., 2017; Weiss and Hennet, 2017). An unbalanced gut microbiota can provoke digestive disorders such as constipation (Fouhse et al., 2016; Liao and Nyachoti, 2017).

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Controlling the feed of the sows is the obvious method to limit digestive disorders. In this context, plant extracts offer an attractive alternative to antibiotics because of their variety of secondary metabolites inducing physiological effects on the gut and microbiota (Acamovic and Brooker, 2005). Among plant extracts, the citrus extract contains many compounds, which have showed effects on the gut and its microbiota: First, pectic oligosaccharides derived from pectin present in citrus fruit promote bifidobacteria and lactic bacteria growth and are well known to inhibit pathogenic bacteria invasion into the gut (Gómez et al., 2014). Furthermore, Unno et al. (2015) demonstrated that citroflavonoids promote the production of short-chain fatty acids in the colon, which results in pH diminution and pathogenic bacteria inhibition. Second, at least one of the citroflavonoid (Hesperidin) affects the gastrointestinal motility (Mendel et al., 2016). Taken together, these results suggest that compounds of the citrus extract are active on the gut and its microbiota.

However, data on the effect of citrus extract on zootechnical performances and welfare are sparse, especially during the critical period of *peripartum*. This study aims to assess the effect of a commercial Standardized Natural Citrus Extract (SNCE) supplementation around farrowing on either sows and piglets' health and zootechnical performances.

MATERIALS AND METHODS

This study was carried out in strict accordance with the recommendations set out in the European Guidelines for accommodation and care of animals (Directive 86/609/CEE).

Instruments and Reagents

SNCE extract (Nor-Spice AB, Nor-Feed SAS, France) was supplied by Nor-Feed SAS Company. Catechin hydrate (>98% high-performance liquid chromatography) from Sigma–Aldrich was used as standard for SNCE total polyphenols content determination. Sabouraud medium, for prebiotic effect determination, were supplied by Laboratoire Humeau. *Lactobacillus acidophilus* strain R52 was also used for *Lactobacillus acidophilus* growth experiments. All spectrophotometer analyses were performed using Cary 60 UV-vis analyzer (Agilent Technologies). DNA from feces samples were extracted with the ZR-96 Soil Microbe DNA kit (Zymo Research, Irvine, CA) according to the manufacturer description. Polymerase chain reaction (PCR) for DNA sample amplification were done using Illumina MiSeq System (Illumina).

Animals and Diets

Fifty sows (Large-White × Landrace), obtained from a commercial farm in France, were randomly divided into two groups: a control (CTL) group (23 sows) with a standard diet (Table 1) and a SNCE group (27 sows) with a standard diet supplemented with SNCE at 2,500 ppm. The SNCE (Nor-Spice AB, Nor-Feed SAS, France) is a 100% natural feed additive based on citrus extract and standardized in terms of active compounds like pectic oligo saccharides and citroflavonoids. Supplementation occurred 10 d before and 5 d after farrowing. Sows were fed using an automatic liquid feeding system following a theoretical delivery curve set-up in its system (Fig. 1). This system adapts the feed delivery to each sow, according to the sows' appetite, in order to avoid feed waste. All animals were fed with the same feed before the beginning of the trial. Sows were reared on slatted floors at temperatures between 20 and 25 °C. Both *multiparous* and *pre partum* sows were selected for this trial. The breeders followed a prophylactic set up in the commercial farm which is described in Fig. 2.

SNCE Total Polyphenols Content

The total polyphenols content was determined following the same UV-spectrophotometric method described in the annexe 1 of the regulation EC N° 2017/307 (COMMISSION IMPLEMENTING

Table 1. Composition of standard diet fed to sows from 10 d before and 5 d after farrowing

Raw material	Weight	%
Barley	657	65.7
Sunflower cake	100	10
Breadcrumbs	100	10
Rape oil cake	70	7
Oat pod	30	3
premix 2.4%[1]	24	2.4
Sodium carbonate	12	1.2
Soya oil	5	0.5
Lysine	2	0.2
Total	1,000	100

[1]Vitamin and mineral premix supplied per kilogram of diet: amino acid lysine (0.71%), methionine (0.25%), threonine (0.49%), tryptophan (0.16%). Vitamins: Vitamin A: 15,000 UI, Vitamin D3: 2,000 UI, Vitamin E: 200 UI. Phytase: 500 phytase unit (FTU), micronutrient: chelate ferrum (80 mg/kg), chelate copper (7 mg/kg), chelate manganese (20 mg/kg), chelate zinc (40 mg/kg), magnesium (0.191%), sodium (0.292%), calcium (0.853%), and phosphorus (0.642%).

REGULATION (EU) 2017/307 of 21 February 2017 concerning the authorization of dry grape extract of *Vitis vinifera* spp. *vinifera* as a feed additive for all animal species except for dogs, 2017). Briefly, 1 g of SNCE was diluted in 50-mL demineralized water. After 5 min of sonication bath (S30H, Elmasonic, Germany), 3 mL of the extract were diluted in 47 mL of demineralized water. Absorbance measurements were taken at 280 nm. Results are expressed in percentage of catechin equivalent. Samples were analyzed in triplicate.

SNCE Effect on Lactobacillus *Growth*

The effect of SNCE on *Lactobacillus acidophilus* growth has been monitored following an adapted method from Mandalari et al. (2007). Briefly, 1 g of SNCE was solubilized in 10 mL of sterilized water. Then, 200 µL of the solution were added to 10 mL of Sabouraud medium which were seeded with 5×10^5 *Lactobacillus acidophilus* per milliliter. The *Lactobacillus acidophilus* growth was assessed by absorbance reading at 650 nm (A_{650} nm), 24 h after SNCE supplementation. The SNCE effect on *Lactobacillus acidophilus* growth was determined comparing A_{650} nm of Sabouraud medium containing SNCE with Sabouraud medium without supplementation. A medium with SNCE was also analyzed in order to check the non-contamination of the SNCE. Each analysis was done in duplicate.

Zootechnical Performances

Sows' feed intake was measured daily by the automatic device for feed distribution available on the farm. Sows were monitored every 2 h. The interval between farrowing and the first observed dejection was registered. Concerning piglets, 20 litters from the SNCE sows group and 14 litters from the CTL sows group were analyzed for this study. When needed, piglets' adoptions were made within the same group. Piglets were weighed per litter at 24 h and 7 d after farrowing. The average weight of each litter and the piglet litters' average weight gain between 24 h and 7 d were calculated.

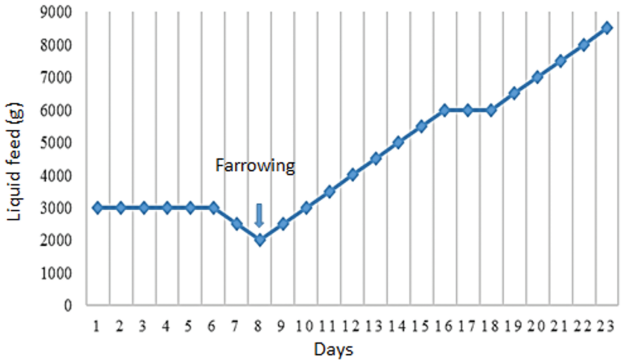


Figure 1. Theoretical delivery curve of feed (g) in this study. Sows were fed using an automatic liquid feeding system following this theoretical delivery curve. This system adapts the feed delivery to each sow in order to avoid feed waste.

Sampling Procedure

At the end of the study, 5 d after farrowing, eight sows from each group (CTL group and SNCE group) were randomly selected for the feces microflora analysis. Rectum content from each sow was collected aseptically and kept in an air tight jar at −20 °C until use.

Microbiota Analysis

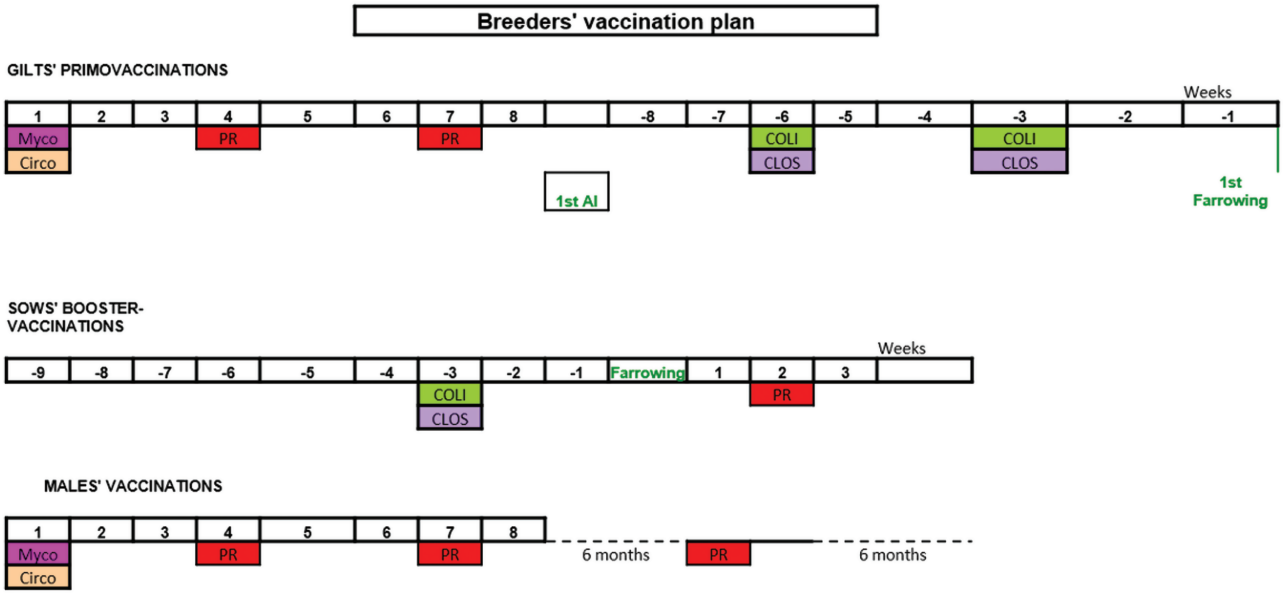
Fecal microbiota was analyzed using 16S ribosomal DNA sequencing following the method described by Verschuren et al. (2018): 60 mg of each feces sample were lyzed, extracted, and purified. The V3–V4 regions of gene coding for 16S rRNA were amplified with the primers F343 (CTTTCCCTACACGACGCTCTTCCGATCTT ACGRAGGCAGCAG) and R784 (GGAGTTCAG ACGTGTGCTCTTCCGATCTTACCAGGGTA TCTAATCCT), Illumina MiSeq technology. Thirty amplicon cycles were performed using an annealing temperature of 65 °C.

All sequences obtained were multiplexed with a 6 bp index which was added to R784 primer. A second 12 cycle PCR was done using forward primers (AATGATACGGCGACCACCGAGATC TACACTCTTTCCCTACACGAC) and reverse primers (CAACAGAAGACGGCATACGAGAT-index-GTGACTGGAGTTCAGACGTGT). Sequences from this second PCR were purified and loaded onto Illumina MiSeq cartridge according to the manufacturer directives. Then, each sequence was assigned to its sample, assembled with the help of Flash software, and clustered using Usearch (Edgar, 2013).

Results are expressed in Operational Taxonomic Unit (OTU) which is an approximation of bacterial species (Nguyen et al., 2016). The affiliation of the OTUs were determined with Usearch V11.0.667 on the RDP dataset16.

Statistical Analyses

Statistical analyses of zootechnical performances were performed by Student's test (*t*-test) or Wilcoxon test, when data were nonparametric.



code	Commercial name	Diseases
PR	Parvoruvax®	Parvovirus and Erysipelas
COLI	Suiseng ®	Neonatal colibacillosis and Clostridium
CLOS	Suiseng ®	Neonatal colibacillosis and Clostridium
Myco	Porcilis Mhyo ID once ®	Mycoplasma hyopneumoniae
Circo	Porcilis PCV®	PMWS (Circovirus)

Figure 2. Prophylactic program set-up during this study.

Shapiro–Wilk normality test was performed to determine whether data were parametric or not. Statistical significance was considered at $P < 0.05$. All statistical analyses concerning zootechnical performances were performed using GraphPad Prism 7 (GraphPad Software). The OTU table of abundance was analyzed by discriminant analysis using principal component (Jombart et al., 2010) and blasted using the NCBI 16S ribosomal RNA sequences database. Similarity between DNA samples from sows were monitored using Bray–Curtis dissimilarity. Statistical analysis of results from sequencing were performed using R software (R Development Core Team, 2008). The package "Biostrings" was used to import fasta files. Phyloseq used to rarefy data. Dunn.test package was used for multiple comparison and ape package was used for phylogenetic data exploration. Vegan and Adegenet packages were used for non-metric multidimensional scaling analysis. Ggplots2 package was used for general plot.

RESULTS

SNCE Total Polyphenols Content

The total polyphenols content of SNCE was 10.22% ± 0.63 catechin equivalent.

Effects of SNCE Dietary Supplementation on Sows

The effect of SNCE on sows can be visualized in Figs. 3 and 4. Numerous variables are significantly affected, namely, feed intake, delay before first excretion after farrowing, microbial communities, and the load of *Lactobacillus*.

SNCE affects the zootechnical performances of the sows. Sows supplemented with SNCE in their diet (8,540 g/d, $N = 27$) had a higher feed intake compared with control sows (7,937 g/d, $P < 0.01$, $N = 23$). The delay between farrowing and first dejection was also significantly lower in the SNCE (1.35 d, $N = 27$) group than in the CTL group (1.88 d, $P < 0.05$, $N = 23$). No mortality was observed during the test period.

SNCE also affects the microbiota of the sows, but the effect was moderate. Indeed, the discriminant analysis was able to separate the two microbial communities of the sows that received SNCE using 21 OTUs (area under the ROC curve = 96%; Fig. 5) with a sequencing depth of 24,830 sequences. Out of the 21 discriminating OTUs, 4 belonged to the *Lactobacillaceae* and 9 belonged to the *Clostridiaceae*. Interestingly, the abundance of

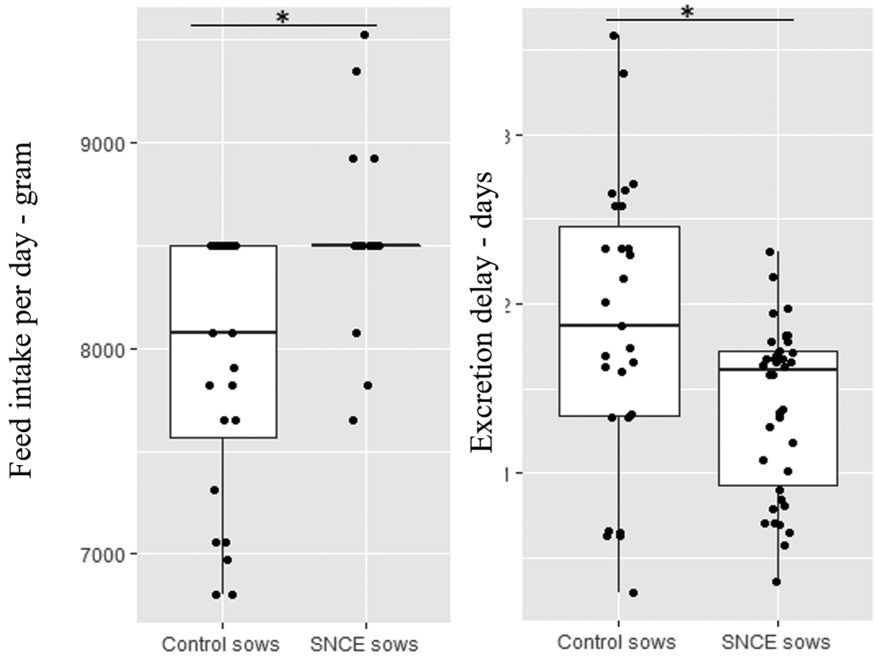


Figure 3. Effect of the Standardized Natural Citrus Extract on feed intake and excretion delay of the sows. The *t*-test was performed to compare the excretion delay of the two treatments. The Wilcoxon test was realized to compare the feed intake per day. The *P*-values between the groups for feed intake and excretion delay was 0.0017 and 0.011, respectively.

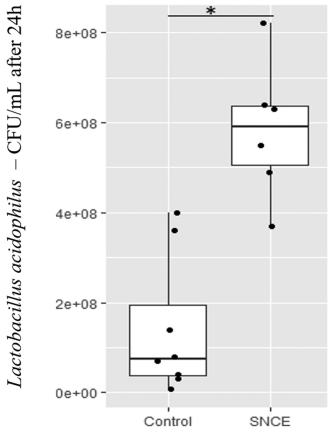


Figure 4. Ability of the Standardized Natural Citrus Extract on the provide substrate for the growth of *Lactobacillus acidophilus* in vitro. The *t*-test was performed to compare the effect of the two treatments ($P = 0.0002$, *t*-test).

Lactobacillus measured by culturing on selective medium was also increased threefold (from $1.41 \pm 0.54 \times 10^8$ to $5.83 \pm 0.62 \times 10^8$ CFU/mL with SD, $P < 0, 05$). The other discriminating OTUs belonged to the *Enterobacteriaceae*, the *Streptococcaceae*, the *Porphyromonadaceae*, and the *Sphingobacteriaceae* (Table 2). However, the effect of the SNCE on microbiota was moderate because none of the OTUs was significant when taken separately and the Bray–Curtis distance between groups was not statistically significant ($P = 0.124$; Fig. 5). Unsurprisingly, the impact of the SNCE was also not visible at the phylum level. The *Firmicutes* represented 87% of the sequences while the *Bacteroidetes* were the second most abundant phylum (5% of the sequences). The SNCE also had no impact on the richness, both groups had similar Chao and Shannon diversity indexes, respectively, 1,373 ± 266 and 3.9 ± 0.2.

Repercussion on the Piglet Performances

The average weight of the litter across 50 sows are available on Table 3. Crucially, feeding the sows with SNCE had an impact on their piglets. In particular, SNCE increased their average weight gain between 24 h and 7 d after birth ($P = 0.02$, Fig. 6). This significant difference became a trend at 21 d ($P = 0.051$, $N = 20$). Regarding the litter size of piglets, statistical analysis showed significant difference between the litter size of piglets from the SCNE group (15.20 ± 3.33) and those from the CTL group (17.56 ± 2.85; $P = 0.0178$, *t*-test). The average born-alive piglet was 15.89 ± 2.99 for piglets from the CTL group and 13.84 ± 2.95 for piglets from the SNCE sows without statistical difference ($P = 0.184$, *t*-test). Also, no difference was observed on *peripartum* after mortality of piglets from SNCE group (1.84 ± 2.10) and CTL group (1.45 ± 2.06).

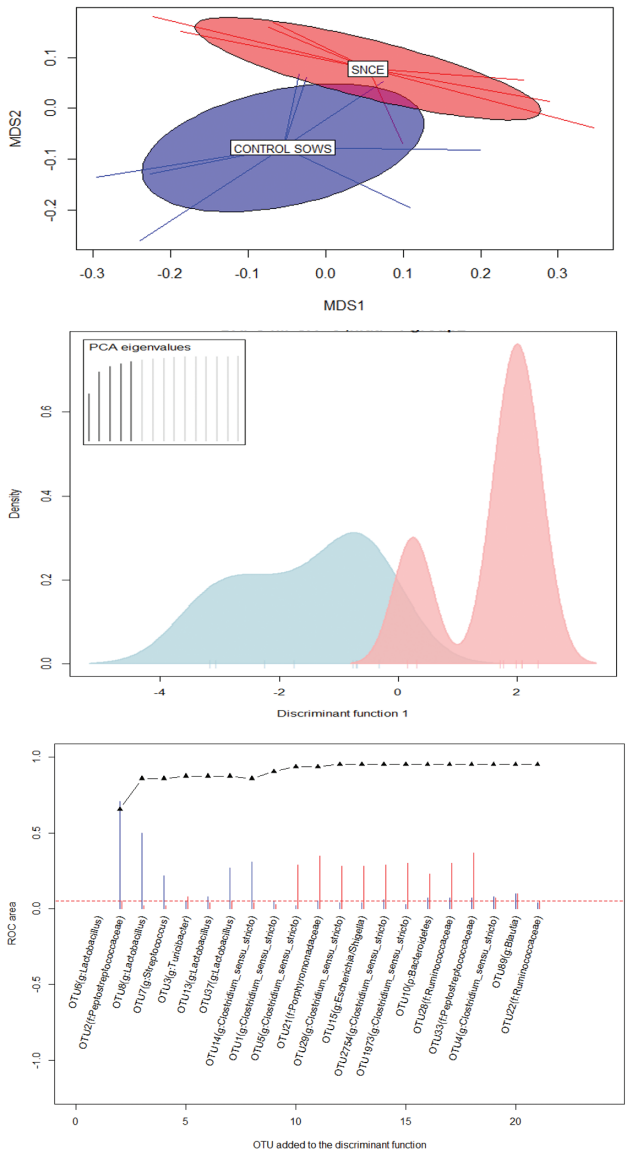


Figure 5. Impact of Standardized Natural Citrus Extract on the fecal microbial communities collected from the sows, collected 5 d after farrowing via non-metric multidimensional scaling (top) and discriminant analysis using principal component with five principal components capturing 94% of the variability (middle) and the contribution of the OTUs to the correct group affiliation and receiver-operating characteristic area (bottom). The stress is 0.088 and *P*-value evaluated by the ADONIS procedure is 0.093 (Top), while the *a*-score function results in *P*-values of 0.04 and 0.05 for the SNCE (blue) and control sows (red) are 0.04 and 0.05, respectively (bottom).

DISCUSSION

Effects of SNCE Dietary Supplementation on Sows

Dietary supplementation of SNCE during *peripartum* allows sows to increase feed intake. These results do not agree with the findings of Cerisuelo et al. (2010), who demonstrate a decrease of feed intake with citrus-based product supplementation at 50 and 100 g/kg on pigs. Other authors also show a decline of feed intake from pigs supplemented with citrus-based product at 10, 20, and 40 g/kg (Baird et al., 1974) and at a dosage between 50 and 150 g/kg (Moset et al., 2015). We used 2,500 ppm but in addition to the dose difference, these discrepancies may be explained by the origin of the tested citrus. In fact, citrus product available on the market can be composed of a unique sort of citrus or a combination of them. In accordance to this, the active compounds vary substantially, which may lead to a large variability of efficiency of the product (Moset et al., 2015). Standardization of these extracts is primordial in order to guarantee their effect independently from the period at which the citrus is collected. The SNCE evaluated here is standardized not only by its manufacturing process but also in terms of concentration of some active compounds such as total phenolic compounds or some citroflavonoids (Hesperidin, Eriocitrin).

In the present study, the SNCE had a positive impact on the sow's welfare by reducing constipation. Constipation is a recurrent phenomenon in *peripartum* period in pig production. After 10 d, all sows had a normal transit but monitoring the interval between farrowing and first dejection is a good way to evaluate the intestinal transit recovery. According to Oliviero (2010), two consecutive days without feces production is synonym to dysbiosis and constipation. In this study, the delay between farrowing and first dejection of the two groups was under 2 d, reflecting a good intestinal transit recovery. However, SNCE supplementation still significantly reduces this delay, possibly because of citroflavonoids that SNCE contain, namely hesperidin and naringenin. In fact, Mendel et al. (2016, 2017) showed that Hesperidin can possess a myocontractive action on porcine intestines in a concentration-dependant matter. Moreover, the naringenin contained in citrus product has been shown to have a laxative effect (Yin et al., 2018). Although SNCE supports the in vitro growth of *Lactobacillus*, a genus with demonstrated effects on intestinal transit (Husebye et al., 2001), the sequencing data does not support this hypothesis.

Sequencing showed a high proportion of *Firmicutes* (87% of the sequences) with a lower proportion of *Bacteroidetes* (5% of the sequences) in the feces. Similar results have already been shown by Guo et al. (2008) on Landrace pigs. Also, freezing the samples before DNA extraction may artificially increase the *Firmicutes* to *Bacteroidetes* ratio (Bahl et al., 2012). In addition, previous research that examined the ZR-96 Soil Microbe DNA kit

Table 2. Average of different species OTU's number from sows' feces samples

OTU ID	RDP genus or family	Blast 1	Counts in SNCE group	Counts in CTL group	*P*-value (uncorrected Wilcox)	Impact on health according to literature
OTU29	*Clostridium_sensu_stricto*	*Clostridium chartatabidum* strain 163 (99.7%)	138.875	69	0.60584122	−
OTU4	*Clostridium_sensu_stricto*	*Clostridium sardiniense* strain DSM *600 AB*161371	1,072.875	500.25	0.32126697	−
OTU14	*Clostridium_sensu_stricto*	*Clostridium* sp. SH-C10 (100%)	84.75	20.625	0.27659399	
OTU5	*Clostridium_sensu_stricto*	*Clostridium butyricum* strain CDC_51208 (100%)	842.5	557.375	0.1626787	+
OTU8	*Lactobacillus*	*Lactobacillus reuteri* strain LR199 (100%)	564	283.625	0.167174	+
OTU13	*Lactobacillus*	*Lactobacillus johnsonii* strain DSM 100219 (100%)	84	60.25	0.84729721	+
OTU21	*Barnesiella*	No close cultivable representative	149	23.375	0.26788487	
OTU7	*Streptococcus*	*Streptococcus gallolyticus* strain PUA070 (100%)	530.25	192.625	0.41283373	−
OTU28	*Intestinimonas*	No close cultivable representative	77.5	40.5	0.05923488	
OTU15	*Escherichial Shigella*	*Escherichia coli* MRY15-131 (100%)	50.75	70.5	*0.05923488*	−
OTU1973	*Clostridium_sensu_stricto*	*Clostridium tertium* strain 372 (98%)	1.75	38.875	*0.01407892*	
OTU89	*Blautia*	*Blautia wexlerae* strain AUH-JLD56 (99.7%)	13.375	61	*0.07469235*	+
OTU10	*Parapedobacter*	No close cultivable representative	123.5	185.125	*0.8472046*	
OTU33	*Clostridium_XI*	*Clostridiales* bacterium 80 (99%)	169.125	246.75	*0.07445496*	−
OTU6	*Lactobacillus*	*Lactobacillus amylovorus* strain HUMB07375 (100%)	449	560	*0.32377055*	+
OTU2754	*Clostridium_sensu_stricto*	No close cultivable representative	800.125	921	*0.73612126*	
OTU37	*Lactobacillus*	*Lactobacillus delbrueckii* subsp. *bulgaricus* strain BCS113 (98%)	11	132.75	*0.02064994*	+
OTU3	*Turicibacter*	*Turicibacter* sp. H121 (100%)	809.625	1,056.75	*0.02064994*	
OTU2	*Clostridium_XI*	*Terrisporobacter petrolearius* strain LAM0A37 (99%)	2,842.875	3,199.25	*0.23587001*	No data available
OTU1	*Clostridium_sensu_stricto*	*Clostridium saudiense* strain JCC (98%)	4,959.875	5,520.375	*0.96157949*	−
OTU22	Unclassified Ruminococcacea	No close cultivable representative	65.875	38.5	*0.38618503*	

CTL group: sows fed with a standard diet. SNCE group: sows fed with a standard diet supplemented with 2,500 ppm of SNCE (i.e., 10 mg/kg body weight). Gray color represents the most abundant OTUs which proportion were enhanced in the SNCE group. White color represents the most abundant OTUs whose proportion was enhanced in the CTL group. Counts from each group represent the average OTU's number of the different analyzed species. Difference is significant when P-value < 0.05.

(Zymo Research, Irvine, CA) used for DNA extraction reported very good sensitivity of the *Firmicutes* phylum (Henderson et al., 2013; Wagner Mackenzie et al., 2015).

The discriminant analysis carried out using the 21 most abundant OTUs allowed to separate microbial communities of SNCE supplemented sows from control sows. Among 21 of the most discriminating OTUs, 4 of them are from the *Lactobacillaceae* and 2 belong to *L. acidophilus* (Du Plessis and Dicks, 1995). However, the OTU abundances did not differ significantly between the groups. Results obtained are not in agreement with the findings of a study by Noh et al. (2014) demonstrating a significant effect of citrus pulp extract supplementation on fecal total anaerobic bacteria population of weanling pigs. The number of samples analyzed (eight per group) may explain the difference between our results and literature.

It is important to notice that some discriminating OTUs enhanced by SNCE belong to taxonomic groups known for their positive effect on gut health and microbiota. For example, the probiotic effect of *Lactobacillus reuteri* has already been shown on piglets. In fact, Chang et al. (2001) demonstrate a decrease of enteric pathogenic bacteria number

Table 3. Average weight of litter 24 h, 7 d, and 21 d after farrowing

Litter number	Group	Average weight of litter (kg) 24 h after farrowing	Group	Average weight of litter (kg) 7 d after farrowing	Group	Average weight of litter(kg) 21 d after farrowing
1	CTL group[1]	1.286	CTL group	2.538	CTL group	5.75
2	CTL group	1.165	CTL group	3.62	CTL group	6.29
3	CTL group	1.7	CTL group	2.071	CTL group	6.29
4	CTL group	1.197	CTL group	2.592	CTL group	6.09
5	CTL group	1.925	CTL group	2.704	CTL group	5.75
6	CTL group	1.535	CTL group	2.567	CTL group	6.21
7	CTL group	1.312	CTL group	2.02	CTL group	5.96
8	CTL group	2.038	CTL group	2.832	CTL group	4.60
9	CTL group	1.977	CTL group	2.545	CTL group	6.42
10	CTL group	1.506	CTL group	1.756	CTL group	3.92
11	CTL group	1.838	CTL group	2.517	CTL group	5.96
12	CTL group	1.529	CTL group	2.375	CTL group	6.17
13	CTL group	1.789	CTL group	2.139	CTL group	3.92
14	CTL group	2.205	CTL group	3.727	CTL group	6.42
15	SNCE group[2]	0.982	SNCE group	2.4	SNCE group	6.52
16	SNCE group	1.55	SNCE group	3.154	SNCE group	5.91
17	SNCE group	1.292	SNCE group	2.645	SNCE group	6.52
18	SNCE group	1.121	SNCE group	2.627	SNCE group	5.90
19	SNCE group	1.208	SNCE group	2.29	SNCE group	5.90
20	SNCE group	0.992	SNCE group	2.83	SNCE group	6.50
21	SNCE group	0.957	SNCE group	2.255	SNCE group	4.13
22	SNCE group	1.01	SNCE group	2.269	SNCE group	5.88
23	SNCE group	1.12	SNCE group	2.692	SNCE group	5.91
24	SNCE group	1.567	SNCE group	2.783	SNCE group	6.38
25	SNCE group	1.562	SNCE group	2.717	SNCE group	4.50
26	SNCE group	1.582	SNCE group	2.269	SNCE group	3.69
27	SNCE group	1.457	SNCE group	2.696	SNCE group	4.50
28	SNCE group	1.677	SNCE group	3.058	SNCE group	5.88
29	SNCE group	1.5	SNCE group	3.125	SNCE group	7.04
30	SNCE group	0.787	SNCE group	1.85	SNCE group	4.13
31	SNCE group	1.42	SNCE group	3.2	SNCE group	6.86
32	SNCE group	1.344	SNCE group	1.925	SNCE group	5.60
33	SNCE group	1.207	SNCE group	2.865	SNCE group	6.86
34	SNCE group	1.677	SNCE group	3.458	SNCE group	7.04

[1]CTL = Control.

[2]SNCE = Standardized Natural Citrus Extract.

on piglet feces when piglets feed with *L. reuteri*. *Lactobacillus johsonii* beneficial effect on microbiota and immunity following *Campylobacter jejuni* infection has also been reported (Bereswill et al., 2017). In addition, *Intestimonas* that was in the discrimintating OTUs may affect short-chain fatty acids production if it is *Intestimonas butyriciproducens* (Kläring et al., 2013).

Similarly, SNCE inhibits several OTUs, which are known to have a negative impact on microbiota and health. For example, the relationship between *Clostridium saudiense strain JCC* and obesity has already been described (Angelakis et al., 2014). In addition, *Escherichia coli MRY15-131* is a known pathogen to be colistin resistant (Sekizuka et al., 2017). However, SNCE also inhibits OTU suspected to be beneficial, such as *Blautia* that converts the arctigenin contained in citrus to the sought-after 3′-desmethylarctigenin (Liu et al., 2013) but tends to be lower in the SNCE group. SNCE also tends to decrease *Lactobacillus amylovorus* (although not significantly) and *Lactobacillus delbrueckii*, which are used as probiotics (Omar et al., 2013; Yu et al., 2013).

Repercussions on the Piglet Performances

Piglets from sows fed with 2,500 ppm of SNCE tend to be heavier than the piglets from

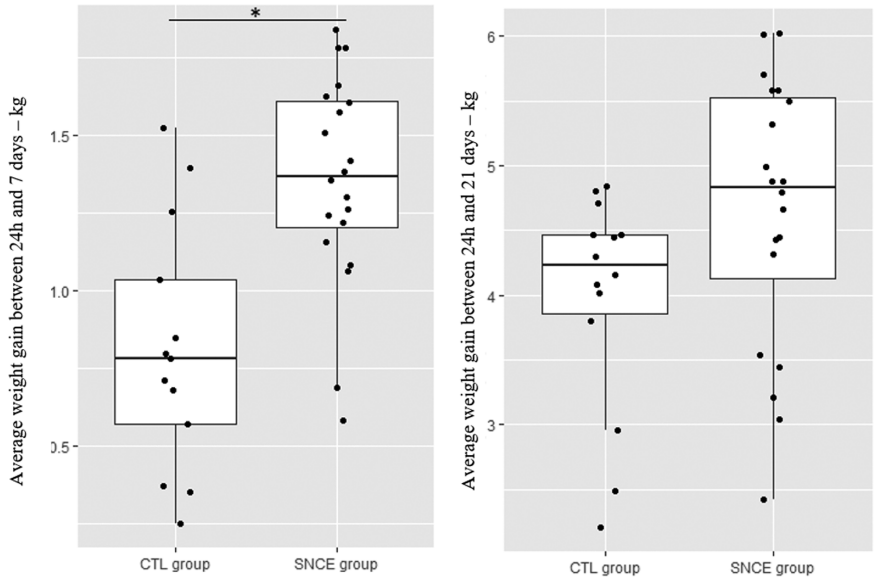


Figure 6. Consequence of maternal programming on the piglets' weight gain. The *P*-values of the *t*-test are 0.02 and 0.051 (14 litters from control sows and 21 litters from Standardized Natural Citrus Extract sows).

the control group. The earlier establishment of a beneficial microbiota in piglets might partly explain this observation (Leblois et al., 2017). In fact, according to Leblois et al. (2017), transmission of microbiota to the offspring occurs at birth and during lactation. Vaginal and fecal microbiota transfer from sows to piglet has also been shown in previous research (Thum et al., 2012; Starke et al., 2013; Paßlack et al., 2015). Cheng et al. (2019) demonstrated that the transmission of microbes from the sows to their new-born piglets correlates with an increase of zootechnical performances of piglets. In fact, their work showed that new-born piglets fed with maternal fecal microbiota orally administered allowed to increase the average daily gain of the piglet. They also revealed an increase of the concentration of fecal and plasma acetate, butyrate and total short-chain fatty acids on supplemented piglets, compared with the control group. In addition, the impact of the maternal microbiota is shown by the decreased mortality of the piglets inoculated with microbial communities from high-parity sows with a documented history of robust litter characteristics (Niederwerder et al., 2018). In this context, SNCE affects the microbiota of the sow, which then might affect the weight gain of the piglets.

Better weight gain of the piglets from SNCE sows may also be explained by the quality and quantity of milk produced by SNCE sows. Indeed, previous studies have already reported that feed supplementation can affect the composition of colostrum and milk (Farmer and Quesnel, 2009; Laws et al., 2009), especially between 1 and 3 d of age (Cheng et al., 2019). Other studies have already shown the positive impact of colostrum from additive supplemented sows on piglet growth performances (Huguet et al., 2006; Boudry et al., 2008; Sugiharto et al., 2015).

The active compounds of SNCE that might remain in the feces of the sow are unlikely to explain the better weight gain of the piglets from sows fed with the citrus extract. Indeed, this mechanism could be triggered because feeding 5% Citrus pulp to weaning piglets together with *Bacillus subtilis* improved the gain to feed ratio and the total density of anaerobic bacteria (Noh et al., 2014). However, previous studies aiming at feeding iron *via* the maternal feces measured that a piglet ingests 20 g of maternal feces per day (Gleed and Sansom, 1982), so that 2,500 ppm of citrus extract in those 20 g of feces are unlikely to have a measurable impact. Nevertheless, a meta-analysis included 16 trials (additional data) shows that SNCE supplementation of piglets increase the average daily gain by 11.7% and decrease the feed conversion ratio by 6.8%.

CONCLUSION

Data from this study show that SNCE supplementation induces both beneficial effects on *peripartum* sow's welfare and litter zootechnical performances, possibly by modulating the gut microbiota of sows. A microbiota transfer from

supplemented sows to new-born piglets could be done in order to confirm these observations. SNCE sows had a better feed intake than control sows. Moreover, 1-wk piglets from supplemented sows had a higher weight gain even though the difference became minor after 3 wk. SNCE supplementation also permitted to reduce the interval between farrowing and first dejection, which is a good marker of a well-functioning intestinal transit and welfare. In this context of high productivity in which animal welfare issues are more and more considerate, solutions need to be implemented. According to our results, SNCE seems to be an interesting way to improve animal welfare and productivity while reducing medication.

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Conflict of Interest: The authors (S.C., M.E.A.B., A.B., M.F., P.C.) work within the research and development department of Nor-Feed SAS. Nor-Feed SAS commercializes a product called NOR-SPICE AB, which is Standardized Natural Citrus Extract.

Data Availability: 16S rRNA data sets of the 21 discriminating Operational Taxonomic Units generated and analyzed in this study are available from the NCBI database, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA531905/.

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