INFLUENCE OF MATERNAL MICROBIAL COMMUNITIES ON THE MUCOSAL MICROBIOME OF NEONATAL PIGS.

Maradiaga, Nidia, Zeineldin, Mohamed, Aldridge, Brian, Lowe, James
Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign.

ABSTRACT

Colostrum is vital to the newborn pig. Hence, cross-fostering is employed to equalize the number of piglet between litters ensuring colostrum intake for their survival and growth. However, little is known about its impact on the intestinal microbiome of the neonatal pig. Twenty-four piglets were enrolled in the study to determine the influence of maternal microbial communities on the mucosal microbiome of the young pig. Piglets were randomly assigned to 1 of 3 treatments according to colostrum source and postcolostral milk feeding for 21 days, as follow: treatment 1 (n = 8), received colostrum and post-colostral milk feeding from their own dam; treatment 2 (n = 8), received colostrum from foster dam and returned to their own dam for post-colostral milk feeding; and treatment 3 (n = 8), received colostrum and post-colostral milk feeding from foster dam. DNA was extracted from nasal, fecal, and gastrointestinal (GI) tract of the piglets and from colostrum, vaginal, and fecal samples of the sows. Discriminant analysis revealed that bacterial communities varied with biogeographical location in the GI tract, with colon being the most diverse section. *Firmicutes* and *Bacteroidetes* were the dominant phyla in the GI tract of the young pig. Bacterial communities in both maternal colostrum and vaginal samples were significantly associated with those present in the GI tract, feces, and nasal passage of piglets. Treatment did not affect bacterial communities present in the piglet GI tract, however, the bacterial communities present in piglet fecal and nasal samples changed over time. Although

cross-fostering did not impact microbial communities in the piglet, this study suggests an impact of colostrum and maternal influence on the development of the microbiome of the piglet.

Keywords: cross-fostering, colostrum, piglet, microbiome, gastrointestinal

INTRODUCTION

Unlike human infants and puppies, at birth piglets have exceptionally restricted body reserves and scarcely get antibodies prenatally (Decaluwé et al., 2014). They are presented to unexpected changes outside their mom's body, experiencing severe ecological difficulties transitioning from a clean uterine environment into a complex and differing microbial environment. Many internal organs including the gastrointestinal (GI) tract are still relatively immature and not prepared for extra uterine life (Sangild et al., 2013). For this reason, 80% of preweaning mortality takes place during the perinatal period, mainly during the first 3 days of life (Tuchscherer et al., 2000). Being this a reason for great welfare concern and conveying incredible financial misfortunes to the swine enterprise (KilBride et al., 2014). Colostrum is still the only source piglets have to receive nutrients and protection (Decaluwé et al., 2014). This significance has led to the development of different management practices to enhance the amount of colostrum received by each piglet, thus reducing piglet morbidity and mortality. Cross-fostering, the transfer of piglets between dams during the farrowing process is a necessary practice to equalize the number of piglet between litters ensuring colostrum intake for their survival and growth (Kirkden et al., 2013). While these techniques are highly effective in promoting neonatal survival, there is little known about their effects on long term piglet performance.

We know in humans microorganisms are transferred from dam to offspring at parturition and during the neonatal period (Cerf-Bensussan and Gaboriau-Routhiau, 2010). Furthermore, breast milk once considered sterile has been demonstrated to be constant sources of microbes to the newborn gut (Collado et al., 2009; Fernández et al., 2013). In humans, microbes are transmitted in a personalized manner and play a key role in the maintenance of intestinal health and homeostasis, and therefore in the prevention of diseases (Fernández et al., 2013). Recently, associations between intestinal microbiota and increased number of intestinal diseases have been described in humans (de Vos and de Vos, 2012). For instance, crohn's disease (Kaser et al., 2010; Buttót et al., 2015), celiac disease (Nistal et al., 2012; Flass et al., 2015), and increased *Clostridium* difficile infections (Grehan et al., 2010; Khoruts et al., 2010) have been associated with intestinal microbiota. Furthermore, microbiota has also been associated with respiratory diseases (Dickson and Huffnagle, 2015) including influenza (Jamieson et al., 2013). In pigs, microbiota also contributes to the development of the GI microbiota influencing the immune system and playing a casual role in the incidence of diarrhea (Zhao et al., 2015). Xian et al. (2014) reported effects of cross-fostering on cecal microbiota determining differences in microbiota between fostered piglets compared to their biological siblings.

The number of studies determining the impact of cross-fostering on the GI microbiota is limited. It is our intention to increase knowledge in this area and to determine if this management practice could significantly impact the microbiota establishment during the early growing period. In view of the fact that the world is crashing into a post antibiotic era, we are in need of efficient management tools that will reduce the impact of disease without therapy and improve the nutritional needs of an increasing world population. Some important headway can be gained by taking a closer look at the interplay between the immune system, microbiota, and host. With the

help of culture-independent molecular techniques we expect to have a better understanding and assessment of the biodiversity of colostral microbiota and its relationship with the establishment and development of the gut microbiota in the growing pig. We hypothesize that cross-fostering piglets, and the timing of the cross-fostering, influences both the piglet's immune system and its microbiota, which in turn, may have an impact on lifelong performance. Hence, the aim of this study was to determine the influence of maternal microbial communities on the mucosal microbiome of the young pig subjected to cross-fostering.

MATERIALS AND METHODS

Animal Management and Experimental Design

Experimental procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. Twenty-four piglets from 2 litter (12 pigs per litter), vaginally delivered from multiparous dams (White × Large) of the same parity on the same day, were enrolled in the study. Piglets were snatch farrowed at birth and placed in warm boxes under a heating lamp in the farrowing pen next to the sow. Daily physical examination including performance, appetite, and fecal score, were performed individually. Piglets were individually identified (ear tag) and stratified according gender, body weight, and good post-parturient health. Piglets were then randomly assigned to 1 of 3 treatment groups according to the source of colostrum and post-colostral milk feeding for 21 days, as follow: treatment 1 (n = 8), received colostrum and milk from their own dam; treatment 2 (n = 8), were litter exchanged at birth to receive colostrum from a foster dam for 24 – 36 hours and then returned to their own dam for post-colostral milk feeding the subsequent days; treatment 3 (n = 8), were litter exchanged at birth to receive colostrum and post-colostral milk from a foster dam, and they remained with the

foster dam for the subsequent days. Each piglet was allowed to sucked colostrum for equivalent times. The piglets were observed to exhibit vigorous teat sucking and subsequent satiation. No antibiotics were administered to the sows; *E. coli/Clostridium* bacteria vaccine was administered pre-farrowing. At birth, piglets received iron, male piglets were not castrated. None of the piglets were administered antibiotics during the experimental period. All piglets were weight directly after birth and before being euthanized.

Sample Collection

At farrowing nasal and vaginal swabs (Pur-Wraps®, Puritan Medical Products, Gulford, Maine) were collected from each sow for microbiome analysis. Sows were restrained with the use of a snare and a mouth gag in order to collect the nasal samples. Nasal and fecal swabs were collected on day 0 and 21 from each piglet for microbiome analysis, following the same procedure as in the sow.

At day 21 (a common weaning time in the pig industry), a group of 13 piglets were humanely euthanized. After opening the visceral cavity, esophagus and rectum were clamped to avoid spilling of gastrointestinal digesta and thus contamination of other intestinal parts. Immediately after removing the gastrointestinal tract from the visceral cavity, standardized locations of the stomach, ileum and mid-colon (divided into 3 equal parts) were exposed with sterile instruments and luminal contents were collected with a swab. Luminal sites were later rigorously washed several times with sterile phosphate-buffered saline (Mediatech, Inc., Manassas, VA) to remove remains of free floating bacteria and proceed to collect mucosal content. Mucosal contents from the stomach, ileum, colon, middle jejunum, distal jejunum,

proximal jejunum, and duodenum were collected aseptically by scraping off the mucosa using number 20 surgical blades (Bard-Parker, Aspen Surgical™ Products, Caledonia, MI). Mucosal scrapings were collected in cryovials and kept on dry ice until being stored at −20 °C. A 2-cm^2 portion of the tissue was excised and placed in a tube with 5 mL RNA LATER® (Sigma-Aldrich, Saint Louis, MO) for qPCR analysis. Mucosal scrapings, luminal swabs, and tissue samples were snap frozen and then stored at −20 °C. The remainder of the animals (n = 11) were penned together at weaning (day 21) and grown to market weight in pens that only contain study pigs. They were reared in a room with their farrowing cohort and cared by farm staff according to standard practices.

DNA Isolation

Genomic DNA was extracted from 0.25 grams of mucosal scrapings (stomach, ileum, colon, duodenum, and middle, distal and proximal jejunum) and swab tips from fecal, luminal, skin and respiratory samples using the MOBIO Power Fecal DNA Isolation Kit (MO BIO Laboratories, INC., Carlsbad, CA) following the manufacturer's protocol. Samples were homogenized using the Bullet BlenderTM (Next Advance; Averill Park, NY) following the manufacturer's recommendation. Colostrum DNA was extracted using the Qiagen DNA Isolation Kit (Hilden, Germany) according to manufacture guidelines. The DNA concentration was determined with the Nanodrop ND-1000 UV-Vis spectrophotometer (Nanodrop Technologies, Rockland, DE) at wavelengths of 260 and 280 nm to assess the purity of the DNA. Samples with a ratio between 1.9 and 2.15 were considered acceptable (Nanodrop Technical Note). DNA integrity was assessed by running a 2% agarose gel (Sigma-Aldrich, Saint Louis,

MO) with SYBR Safe DNA Gel Stain (Invitrogen, Grand Island, NY). Extracted DNA was stored at −20 °C.

16S rRNA Gene Amplification and Sequencing

16S rRNA genes were amplified using specific primers, F28 (5'-GAGTTTGATCNTGGCTCAG) and V1-V3 R519 (5'-GTNTTACNGCGGCKGCTG), to target the V1-V3 hypervariable region. The PCR products were sequenced using Illumina MiSeqV3 platform (Ilumina, San Diego, CA) sequencing combined with Fluidigm Access Array. Amplification technique was performed at the W. M. Keck Center for Comparative and Functional Genomics (University of Illinois, Urbana, IL). The fluidigm constructed library was quantitated by qPCR and sequenced on one MiSeq flowcell for 301 cycles from each end of the fragments using a MiSeq 600-cycle sequencing kit (version 3). Fastq files were generated and demultiplexed with the bcl2fastq v1.8.4. Conversion PhiX DNA was used as a spike-in control for MiSeq runs.

Phylogenetic Assignment and Processing of Sequenced Reads

All the total reads obtained from the sequences [230 samples; 23,870,950 reads, as follow: fecal samples generated 1,811, 829 sequences (median = 69,987; range=5,288-192,566), respiratory samples generated 1,388,305 sequences (median = 63,801; range= 4,288-156,835), intestinal samples generated 8,816,931 sequences (median = 375,529,094; range= 15,025-183,367), and sow samples collected generated 379,752 (median = 61,399; range= 6,661-90,947)

sequences], were processed together using Illinois Mayo Taxon Organization from RNA Dataset Operations (IM-Tornado; v 2.0.3.2) (Jeraldo et al., 2014) to generate Operational Taxonomical Units (OTU), and subsequently clustered into 52,6419 OTUs based on 97% similarity using Greengenes as a reference database. Following sequencing, 16S rRNA gene reads were assessed for quality, only reads that were longer than cutoff lengths were processed for OTU picking. Quality scores were generated using Fast QC. All reads were initially 300 bases long, the number of bases covered by read1 and read2 were longer than the fragment length and therefore there was partial overlap between read1 and read2. To be able to run IM-TORNADO's regular pipeline, this overlap needed to be removed. For this, trimmomatic (http://www.usadellab.org/cms/?page=trimmomatic) was run to trim primer sequences at the 5' end and then cropped read1 to 250 and read2 to 200 bases long so that there will not be any overlap between read1 and read2. All the data preprocessed as described above was run with IM-TORNADO for the regionV1V3.

Diversity Indices

Alpha diversity analysis was run to know diversity within the samples or categories, while beta diversity analysis was run to determine diversity between samples or groups. The OTU table was generated by IM-TORNADO pipeline and the mapping file was required for this analysis. Quantitative insights into Microbial Ecology (QIIME; http://qiime.org/) was used to first convert Biological Observation Matrix (BIOM) file to text format file, and create a summary of OTU table generated by IM-TORNADO to finally run alpha and beta diversity analysis scripts. Alpha diversity at several different rarefactions was calculated and then the

results from all different rarefactions were collated, and then plotted as alpha rarefaction plots by QIIME. By default, the minimum rarefaction depth is 10 and the maximum is median sequence over sample count. These defaults are useful for determining whether you have enough depth in your samples to accurately capture all the diversity present. If the lines eventually plateau, then there is enough depth, otherwise the samples may not have enough depth to capture all possible OTUs present. QIIME was used to calculate beta diversity values and create a distance matrix that could be later visualized. Chao1, observed species, PD whole tree and Shannon diversity index were calculated with QIIME.

Statistical Analysis

Statistical analyses of bacterial communities were performed using JMP 12.2 (SAS Institute Inc., Cary, NC). The relative abundance of fecal bacterial taxa for each treatment groups were compared using general linear models (ANOVA). The model for bacterial communities in the piglet GI tract contained the fixed effect of treatment and GI site. The model for bacterial communities in piglet nasal and fecal samples contained the fixed effects of treatment and time. The relative abundances of different bacterial taxa in each sample as covariates and sampling days as the categorical variable were used in stepwise discriminant analysis as described by Zinicola et al. (2015). In this way the microbial shift from day 1 until day 21 was illustrated using canonical loading plots. In our study, variables were removed in a stepwise manner until only variables with a $P > 0.001$ were retained in the final model. Fastq data obtained as results of sequencing samples of sows and piglets were uploaded to the sequence read archive (SRA) on National Center for Biotechnology Information (NCBI) web page tool

(http://www.ncbi.nlm.nih.gov/sra) to make the files available for a public database (BioProject ID accession number PRJNA319360). To assess the association between bacteria genera, present in colostrum, vaginal, and fecal samples of the sow with bacteria genera present in the GI tract, fecal, and nasal samples of the young pig regression and correlation analyses were performed using the REG and CORR procedures of SAS (v9.4 Institute Inc., Cary, NC). Statistical significance was declared at $P \leq 0.05$ and trends toward significance effects were noted when $0.05 < P \leq 0.10$.

RESULTS

Microbial Diversity

Reads were pooled and analyzed using various diversity metrics for each group to calculate diversity of microbial communities. The detailed diversity estimates can be found in Table 2.1. Metrics used were Chao1 index of microbial richness, observed species, and Shannon index of biodiversity. The diversity indices used represent how many different taxa were present in a sample, higher numbers indicate higher diversity. Indices of biodiversity showed higher number of microbial communities in the colon section of GI tract and in treatment 1 (Figure 2.1). Additionally, fecal and nasal samples of day 21 showed higher microbial diversity compared to samples of early piglet age (Figure 2.2).

Relative Abundance of Bacterial Phyla in the Gastrointestinal Tract of Young Pigs

The OTUs were classified into 18 bacterial phyla, of these, 5 phyla were ≥ 1%. Comparison of relative abundance at the phylum level revealed that the major phyla dominating the microbiome were *Firmicutes* 64%, *Bacteroidetes* 16%, *Proteobacteria* 12%, *Spirochaetes* 4%, and *Fusobacteria* 1% (Figure 2.3). No differences ($P > 0.05$) on the aforementioned phyla were found between treatments. However, their relative abundance was different ($P < 0.05$) among GI sites (Figure 2.3). *Firmicutes* were highly abundant through the GI tract. Greater relative abundance of *Bacteroidetes* was found in the colon compared to the other GI sites. *Proteobacteria* was observed along the GI tract with no significant differences ($P > 0.05$) between sites. Although statistical differences were not observed among GI sites, reduced relative abundance of *Proteobacteria* was observed in colon. *Spirochaetes* and *Fusobacteria* were not as predominant compared to the others abovementioned bacteria phyla. However, relative abundance of *Spirochaetes* was predominant in colon, especially in that of treatment 3.

Relative Abundance of Bacterial Phyla in Fecal Samples of Young Pigs

Relative abundance of bacterial phyla in fecal samples was not affected ($P > 0.05$) by treatment (Figure 2.4). However, relative abundance changed ($P < 0.05$) over time. The relative abundance of *Firmicutes* was predominant at day 0 but abruptly decreased at day 21. The opposite occurred with *Proteobacteria*, *Spirochaetes*, and *Bacteroidetes* which relative abundance was lower at day 0 but significantly increased at day 21.

Relative Abundance of Bacterial Phyla in Nasal Samples of Young Pigs

In nasal samples, relative abundance of bacterial phyla was not affected ($P > 0.05$) by treatment (Figure 2.5). However, relative abundance changed over time ($P < 0.05$). *Firmicutes* was highly present at day 0, but significantly decreased at day 21. The opposite occurred for *Proteobacteria,* which had lower relative abundance at day 0 but significantly increased at day 21. Similar tendency was observed for *Bacteroidetes*, although, this phylum was not as abundant as *Proteobacteria* at day 21. As we went deeper in taxonomy, we were able to see greater variation between sites, successively all samples were assessed at a genus level.

Abundance of Bacterial Genera Present in the Sows: Colostrum, Vaginal, and Fecal Samples

Bacterial communities present in colostrum, vaginal, and fecal samples collected from the sows were classified into 104 predominant bacterial genera, of which, 23 for colostrum, 21 for vaginal, and 17 for fecal were ≥ 1% (Figure 2.6). *Lactobacillus* and *Clostridium* were highly abundant in colostrum (38 and 24%, respectively) and vaginal (52 and 13%, respectively) samples. Similarly, in fecal samples *Lactobacillus* (56%), *Campylobacter* (7%), and *Anaerococcus* (5%) were the predominant genera. A large number of bacteria genera (*Allobaculum, Aminiphilus, Anaerovorax, Anoxynatronum, Barnesiella, Butyricicoccus, Butyricimonas, Cloacibacillus, Coprococcus, Corynebacterium, Escherichia/Shigella, Eubacterium, Finegold, Flavonifractor, Gilvibacter, Hydrogenobaculum, Oscillibacter, Peptoniphilus, Phascolarctobacterium, Porphyromonas, Prevotella, Pseudoflavonifractor, Pseudomonas, Ruminococcus, Saccharofermentans, Staphylococcus, Streptococcus, Succinivibrio, Syntrophaceticus, Tannerella, Turicibacter, Veillonella,* and *Xylanibacter*) were present in less than 5% in colostrum, vaginal, and fecal samples (Figure 2.6).

Abundance of Bacterial Genera in the Gastrointestinal Tract Young Pigs

Bacterial communities present in the GI tract of young pigs were classified into 178 bacterial genera, of which, 40 were ≥ 1%. Treatment did not have a significant ($P > 0.10$) effect in bacterial communities present in the GI tract, except for *Clostridium*, *Faecalibacterium*, and *Haemophilus* (<1%) that were different ($P < 0.05$) among treatment (Table 2.2). Higher percentage of *Clostridium* and *Haemophilus* were present in the GI tract of treatment 1 and treatment 2 compared to treatment 3, whereas *Faecalibacterium* was higher in treatment 1 compared to treatment 2 and treatment 3 (Table 3). Bacterial communities were different among GI sites ($P < 0.05$), differences between GI sites and treatment are illustrated Figure 2.7. The stomach, duodenum, jejunum, ileum, and colon accounted for 17, 19, 12, 24, and 28% of total bacterial genera present in the GI tract, respectively. *Lactobacillus* and *Clostridium* were the predominant genera in stomach (51 and 11%, respectively), duodenum (61 and 65%, respectively), jejunum (56 and 24%, respectively), and ileum (15 and 34%, respectively). Colon was the most diverse section of the GI tract and no particular predominance of bacterial genera was observed (Figure 2.7). However, *Treponema* was mostly present in colon of treatment 3 (35%) compared to treatment 1 (< 1%) and treatment 2 (1%), and increased in jejunum (10%) and ileum (15%) of treatment 3. *Tannerella* was mostly present in colon (10 – 15%) and ileum (20%) in treatment 3. In a similar way, *Prevotella* was predominant in colon (15 – 20%) meanwhile in the rest of the GI tract it was present < 1%. *Ruminococcus* (8%) and *Sphaerochaeta* (5%) were mostly present in colon. Bacterial communities present in the stomach, jejunum, and duodenum were similar to each other than those present in ileum and colon, as represented in Figure 2.8.

Abundance of Bacterial Genera in Fecal Samples of Young Pigs

Bacterial communities present in fecal samples from piglets at day 0 and 21 were classified 142 genera, of which, 18 were greater than 1%. Bacterial genera in fecal samples other than *Campylobacter* and *Fluviicola* were not affected ($P > 0.05$) by treatment (Table 2.3). However, abundance of these two genera was below 5%. While *Campylobacter* was mostly present in treatment 2 (5%) compared to treatment 1 (< 1%) and treatment 3 (1%), *Fluviicola* was present in treatment 1 (1%) only. Regardless of treatment, bacteria genera predominant in fecal samples were *Lactobacillus* (16%), *Clostridium* (15%), *Treponema* (12%), and *Bacteroides* (10%) with the rest accounting < 10% (Figure 2.9). Bacteria communities in fecal samples changed ($P < 0.05$) over time (Figure 2.9). While *Lactobacillus* and *Clostridium* were the predominant genera at day 0 (34 and 20%, respectively), their abundance decreased to 5% at day 21. On the other hand, *Bacteroidetes* increased from 3% at day 0 to 18% at day 21. Although other bacteria genera (*Butyricicoccus, Campylobacter, Fluviicola, and Turicibacter*) changed significantly from day 0 to day 21, their abundance was less than 5%.

Abundance of Bacterial Genera in Nasal Samples of Young Pigs

Bacterial communities present in nasal samples from piglets at day 0 and 21 were classified into 156 genera, of which, 22 were greater than 1%. Treatment did not have a significant effect ($P > 0.10$) in bacterial communities present in nasal samples, however, bacterial communities changed over time (Table 2.4). While *Clostridium* and *Lactobacillus* were the predominant genera at day 0 (25 and 24%, respectively), their abundance completely decreased to less than 1% at day 21 (Figure 2.10). On the other hand, *Moraxella* increased from

11% at day 7 to 78% at day 21 (Figure 2.10). Although other bacteria genera (*Aerococcus, Aminiphilus, Anaerotruncus, Butyricicoccus, Clostridium, Coprococcus, Escherichia/Shigella, Ethanoligenens, Eubacterium, Facklamia, Flavonifractor, Megasphaera, Oscillibacter, Psychrobacter, Saccharofermentans,* and *Syntrophaceticus Turicibacter*) changed significantly from day 0 to day 21, their abundance was less than 5% (Figure 2.10).

Association between Bacterial Genera Present in the Sow and in the Young Pigs

Bacterial genera present in the GI tract of the piglet had the highest correlation with bacterial genera present in colostrum (r = 0.93; P <0.0001; R^2 = 0.88), vaginal (r = 0.99; P <0.0001; R^2 = 0.99), and fecal samples (r = 0.96; P <0.0001; R^2 = 0.91) of the sow (Table 2.5). Although lower than the correlations of the GI tract, bacteria genera present in fecal samples of the piglet had high correlation with bacteria genera present in colostrum (r = 0.72; P <0.0001; R^2 = 0.51), vaginal (r = 0.65; P <0.0001; R^2 = 0.41), and fecal samples (r = 0.57; P <0.0001; R^2 = 0.31) of the sow (Table 1). The lowest correlations were observed between bacteria genera present in piglets nasal samples with bacteria genera present in colostrum (r = 0.50; P <0.0001; R^2 = 0.24), vaginal (r = 0.42; P <0.0001; R^2 = 0.17), and fecal samples (r = 0.34; P <0.0001; R^2 = 0.11) of the sow (Table 2). Surprisingly, the highest correlation was between bacteria genera present in vaginal samples of the sow and the bacteria genera present in the GI tract of the young piglet.

DISCUSSION

The influence of gut microbiota in gastrointestinal diseases has successfully been demonstrated through next generation sequencing. Likewise, these techniques have revealed the importance of gut microbiota in animal gut. Clarifying normal bacterial communities versus pathogenic bacteria in the pig is pivotal for establishing differences associated with disease. In our study we used 16S rRNA sequencing to determine if cross-fostering piglets influenced the microbial communities in the developing piglet gut microbiome. The overall goal was to assess the influence of maternal microbial communities on the mucosal microbiome of the young pig subjected to cross-fostering. The current study found that the dominant phyla in the gastrointestinal tract of the young pig were *Firmicutes*, *Bacteroidetes*, followed by *Fusobacteria*, *Proteobacteria*, and *Actinobacteria*. These results are in accord with recent studies indicating the predominance of these particular phyla (Ley, 2008; Poroyko et al., 2010; Schmidt et al., 2011; Schokker et al., 2014). Additionally, prior studies have noted the role of microbes present in the mother responsible of colonizing the gastrointestinal tract of the young pig (Stark and Lee, 1982). Surprisingly, in our study microbial profiles in vaginal, colostrum, and fecal of the sow were similar between each other with a predominance of the genera *Lactobacillus* and *Clostridium*. These genera were also found in high abundance in the gastrointestinal tract of the young pig. A possible explanation for this is that *Lactobacillus* and *Clostridium* were highly abundant in colostrum and vaginal samples of the sow and these may contribute to the colonization of the intestine of the young pig. This finding, while preliminary, suggests there is an influence of maternal colostral and vaginal microbial communities on the mucosal bacterial populations of the GI tract of the young pig. Additionally, influence of maternal microbiome seems to be highest soon after birth, but appears to diminish with time. These results are consistent with those of Mach et al. (2015) where they reported bacterial genera being shared

between sow and piglet supporting the idea of bacterial strains passed down to the offspring via breastmilk. In our study results demonstrated that the composition of fecal and nasal microbiota changed as the pigs aged. Microbial profiles of fecal and nasal samples of newborn pigs were significantly different from older pigs (21 days of age). These results are consistent with those of Thompson et al. (2008) who agreed that bacterial ratio changes with age and more specifically that *Bacteroidetes* in feces increased with age. This matches our results in which we found an increase in *Bacteroidetes* at day 21.

Our results suggest that microbes vary throughout the GI tract, these results are in accord with Isaacson and Kim (2012), microbial communities found in the small intestine (jejunum, duodenum, ileum) were different than that found in the large intestine (colon). Colon was very diverse and no predominance of genera was observed, however, *Treponema* was most abundant in colon specifically in treatment 3. *Treponema* are fastidious and difficult to cultivate, it may be found in vaginal, oral, and GI tract of humans, animals and insects. Recent identification of *Treponema* relies solely on metagenomics techniques (Evans et al., 2011). The finding of *Treponema* in our study can provide some insight on commensal and pathogenic *Treponemas*, although further phylogenetic studies are necessary to elucidate this, and be used as biomarkers for future diseases. According to DiBaise et al. (2008) these locations have different metabolic functions and therefore the microbes vary between locations. For instance, the small intestine is in charge of digestibility and absorption, meanwhile large intestine contains large number of bacteria in charge of fermentation. Furthermore, changes in gut microbiota have been associated with caloric intake and body weight (Park et al., 2014) as well as host genetics (Ley, 2008; Benson et al., 2010). In our study, we did not take into consideration body weight or genetics; it is possible this may contribute to the changes in microbial communities. In contrast to previous

findings (Zhao et al., 2015), *Firmicutes* was more abundant in the small intestine and *Bacteroidetes* was increased in the large intestine. Fecal samples showed higher abundance of *Bacteroidetes* at day 21. It is possible therefore, that feces were mainly representative of the large intestine and not representative of the entire GI tract (Zhao et al., 2015). *Proteobacteria* and *Actinobacteria* were present mainly in nasal samples. In the GI tract, treatment had an effect on *Clostridium*, *Faecalibacterium*, and *Haemophilus*, it is possible therefore, that cross-fostering did impact the trajectory of certain genera in the development of the mucosal microbiome. Although not statistically different some genera were present on treatment 3 that were not present on treatment 1 or treatment 2 such is the case of *Verrucomicrobia*. According to Dubourg et al. (2013), the prevalence of this particular phyla and *Synergistetes* may suggest dysbiosis and a risk to the health of the pig's gut. In our study *Synergistetes* remained steady especially in the colon. Vianna et al. (2007) found that although this phylum is normal microbiota, its high abundance could potentially play a role in periodontal disease. Although in this study we could not determine a difference and/or association between treatments and these two phyla, the prevalence and quantity of these bacteria may be suggestive of a dysbiotic gut.

CONCLUSION

This project is a unique assessment of microbial populations within the context of cross fostering and efforts were made to address possible confounding factors and maintain conclusions within limitations of the experimental design. Overall the results from this study revealed the predominance of the phylum *Firmicutes* and *Bacteroidetes* in the gastrointestinal tract of the young pig, the gastrointestinal tract of the young pig is highly diverse specially the

colon. Most of the variability was noticed at a genus level, meaning that bacteria present in a low quantity could be key microbes to understand the functions of the microbiome and its relationship with disease. Furthermore, there is an influence of maternal microbial populations on the development of the newborn pig and this microbiota continues to change as the pig grows. Changes in microbial communities although not addressed in this study may be caused by a variety of factors, including environment, antibiotic, stress and genetics. The vast majority of gut microbiota studies have focused on the descriptions of the bacteria present in the gut. However, future trials should assess the dynamics of gut microbiota, its translation to function and the effect of these functions on health and well-being. This will likely provide researchers with crucial information that will be used to improve productivity in food animals. The microorganisms present in different GI sites resemble those present in colostrum and vaginal. This allows to hypothesize a strong influence of colostrum and vaginal in the development of the gastrointestinal microbiota of the newborn piglet. Techniques such as metatranscriptomics and metabolomics will be needed to reveal causes and effects of microbial shifts in the gut and could potentially reveal biomarkers of disease before clinical symptoms appear.

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Table 2.1. Microbial diversity estimates

Piglet GI[1] site	Shannon[2]	SD[3]	Chao[4]	SD	Observed sp[5]	SD
Piglet GI site						
Stomach	5.33	0.92	88.0	1.39	86.0	5.87
Duodenum	4.86	0.97	66.3	6.38	74.7	8.81
Jejunum	5.26	0.63	86.9	1.32	68.8	2.37
Ileum	5.31	0.86	100.6	2.23	75.0	3.22
Colon	6.90	0.65	106.5	4.79	135.7	9.96
Piglet						
Fecal	3.76	0.32	100.1	1.25	74.4	3.96
Nasal	4.19	1.52	103.6	0.25	135.6	9.70
Piglet fecal by day						
0	3.78	0.56	65.3	0.52	66.0	7.56
21	4.26	0.76	105.6	3.56	139.0	9.57
Piglet nasal by day						
0	3.13	0.57	28.0	5.23	46.8	8.56
21	4.36	0.60	32.6	6.24	105.6	3.26
Sow						
Colostrum	4.63	0.30	103.7	8.90	120.1	5.20
Fecal	3.79	0.68	172.2	9.60	120.8	10.26
Vaginal	4.31	0.24	126.5	3.23	147.7	8.39
Overall						
Sow	4.05	0.62	157.6	4.56	137.6	8.45
Treatment 1	4.02	1.12	106.8	5.11	81.7	4.65
Treatment 2	3.86	1.12	96.2	5.23	76.5	0.57
Treatment 3	3.86	0.88	92.7	4.56	67.2	1.24
Overall day						
0	4.23	0.50	121.3	1.24	86.2	0.32
21	5.79	1.08	95.2	2.45	173.3	3.78

[1] Gastrointestinal.
[2] Shannon index of biodiversity.
[3] Standard deviation.
[4] Chao 1 index of microbial richness.
[5] Observed species.

Table 2.2. Bacteria genera present in the different gastrointestinal (GI) tract of piglets in treatment 1, 2, and 3

Phylum	Genus	Treatment[1]			SEM[2]	GI Site					SEM	*P*[3]	
		1	2	3		Colon	Duodenum	Ileum	Jejunum	Stomach		Trt	GI site
Bacteroidetes	Alloprevotella	1.31	1.83	0.59	0.34	1.74	0.79	0.31	0.68	3.02	0.52	0.09	0.02
Firmicutes	Anaerostipes	0.16	0.18	0.03	0.06	0.44	0.10	0.07	0.06	0.14	0.09	0.19	0.03
Bacteriodetes	Bacteroides	2.94	1.37	1.74	0.74	5.58	1.86	4.34	1.68	1.22	1.13	0.35	0.05
Bacteroidetes	Bergeyella	1.03	0.80	0.50	0.18	0.25	1.57	0.26	0.61	2.45	0.28	0.19	0.001
Firmicutes	Clostridium	16.2	21.0	4.40	4.05	6.72	7.94	29.1	24.8	12.5	6.18	0.05	0.05
Firmicutes	Dorea	0.36	0.24	0.58	0.17	1.24	0.24	-0.01	0.12	0.21	0.25	0.38	0.02
Firmicutes	Faecalibacterium	0.55	0.09	0.01	0.12	0.67	0.55	0.70	0.39	0.43	0.19	0.03	0.60
Proteobacteria	Haemophilus	0.14	0.26	0.05	0.06	0.02	0.05	0.21	0.09	0.31	0.09	0.05	0.12
Bacteriodetes	Hallella	0.13	0.14	0.14	0.04	0.49	0.00	0.12	0.05	0.01	0.06	0.98	0.001
Firmicutes	Lactobacillus	37.1	32.3	43.4	4.96	4.00	61.9	14.5	51.8	53.4	7.58	0.36	0.001
Proteobacteria	Moraxella	3.19	1.43	2.30	0.75	0.89	5.24	0.89	2.67	6.28	1.15	0.31	0.01
Proteobacteria	Paraperlucidibaca	0.28	0.09	0.10	0.07	0.13	0.24	0.13	0.34	0.58	0.11	0.15	0.04
Bacteriodetes	Porphyromonas	1.04	0.90	1.05	0.20	0.04	1.06	0.04	0.76	3.30	0.31	0.85	0.001
Bacteroidetes	Prevotella	2.24	2.0	2.55	0.63	9.58	0.36	0.44	0.27	0.54	0.97	0.83	0.001
Firmicutes	Ruminococcus	1.54	1.76	2.47	0.73	5.39	0.97	0.83	0.43	0.05	1.11	0.65	0.02
Spirochaetes	Treponema	1.45	0.67	2.02	0.40	5.65	0.14	0.92	0.26	0.26	0.61	0.11	0.0002
Fusobacteria	Streptobacillus	0.07	0.11	0.11	0.03	-0.03	-0.01	-0.03	0.02	0.40	0.05	0.59	0.003
Firmicutes	Streptococcus	1.01	0.99	0.60	0.23	0.17	2.35	0.33	0.85	1.37	0.35	0.40	0.01
Firmicutes	Turicibacter	0.66	0.92	0.61	0.21	0.08	0.60	0.63	0.36	1.61	0.32	0.54	0.03

[1] Treatment: 1 = litter received colostrum and post-colostrum milk feeding from their own dam; 2 = litter exchanged at birth to receive colostrum from a foster dam and then returned to their own dam for post-colostral milk feeding for the subsequent days; and 3 = litter exchanged at birth to receive colostrum and post-colostrum milk feeding from a foster dam, they remained with the foster dam for the subsequent days.[2] Greatest standard error of mean (SEM).[3] Trt = treatment; GI site = gastrointestinal site

Table 2.3. Bacteria genera present in fecal samples of treatment 1, 2, and 3 at day 0 and 21

Phyla	Genera	Treatment[1]			SEM[2]	Day		SEM	*P*[3]	
		1	2	3		0	21		Trt	Day
Bacteroidetes	Bacteroides	2.73	5.10	-2.09	5.09	2.73	17.91	5.09	0.40	0.09
Firmicutes	Butyricicoccus	1.53	1.03	1.00	0.33	1.53	0.35	0.33	0.40	0.06
Proteobacteria	Campylobacter	-0.39	4.56	-0.02	0.47	-0.39	1.31	0.47	0.02	0.06
Firmicutes	Clostridium	19.99	26.46	19.78	1.96	20.0	4.78	1.96	0.15	0.01
Bacteroidetes	Fluviicola	0.46	-0.02	-0.05	0.07	0.46	0.56	0.07	0.04	0.20
Firmicutes	Lactobacillus	34	24	37	5.09	34.3	5.03	5.09	0.30	0.02
Firmicutes	Turicibacter	5.08	5.79	6.44	0.74	5.08	-0.37	0.74	0.46	0.01

[1] Treatment: 1 = litter received colostrum and post-colostrum milk feeding from their own dam; 2 = litter exchanged at birth to receive colostrum from a foster dam and then returned to their own dam for post-colostral milk feeding for the subsequent days; and 3 = litter exchanged at birth to receive colostrum and post-colostrum milk feeding from a foster dam, they remained with the foster dam for the subsequent days.
[2] Greatest standard error of mean (SEM).
[3] Trt = treatment; Day = 0 and 21.

Table 2.4. Bacteria genera present in nasal samples of treatment 1, 2, and 3 at day 0 and 21

Phylum	Genera	Treatment			SEM	Day		SEM	*P*	
		1	2	3		0	21		Trt	Day
Firmicutes	Aerococcus	1.15	0.98	0.85	0.15	1.15	0.17	0.15	0.43	0.02
Synergistetes	Aminiphilus	0.28	0.41	0.38	0.07	0.03	0.01	0.08	0.44	0.04
Firmicutes	Anaerotruncus	0.41	0.45	0.37	0.05	0.41	0.00	0.05	0.50	0.01
Firmicutes	Butyricicoccus	0.24	0.25	0.25	0.01	0.24	-0.01	0.01	0.50	0.01
Firmicutes	Clostridium	25.1	25.2	27.0	2.23	25.05	0.66	2.23	0.76	0.01
Firmicutes	Coprococcus	2.76	3.26	3.91	0.73	2.76	-0.42	0.73	0.54	0.04
Proteobacteria	Escherichia/Shigella	1.08	0.74	1.03	0.21	1.08	0.13	0.21	0.50	0.04
Firmicutes	Ethanoligenens	0.03	0.03	0.03	0.00	0.03	0.00	0.00	0.50	0.01
Firmicutes	Eubacterium	0.34	0.28	0.35	0.05	0.34	0.03	0.05	0.62	0.02
Firmicutes	Facklamia	0.28	0.06	0.39	0.22	0.28	1.84	0.22	0.55	0.01
Firmicutes	Flavonifractor	0.39	0.37	0.48	0.06	0.39	-0.02	0.06	0.50	0.02
Firmicutes	Lactobacillus	23.8	25.3	25.7	1.07	23.8	-0.8	1.07	0.48	0.01
Firmicutes	Megasphaera	1.10	1.48	1.10	0.25	1.10	-0.12	0.25	0.50	0.04
Proteobacteria	Moraxella	10.94	2.14	9.66	9.07	10.9	78.0	9.07	0.73	0.02
Firmicutes	Oscillibacter	0.77	0.99	0.92	0.11	0.77	-0.04	0.11	0.40	0.01
Firmicutes	Psychrobacter	1.20	1.23	1.19	0.03	1.20	-0.002	0.03	0.63	0.01
Firmicutes	Saccharofermentans	0.57	0.71	0.74	0.11	0.57	-0.09	0.11	0.50	0.03
Firmicutes	Syntrophaceticus	0.65	0.78	0.86	0.14	0.65	-0.06	0.14	0.58	0.03
Firmicutes	Turicibacter	3.33	3.18	2.44	0.51	3.33	0.52	0.51	0.46	0.03

[1] Treatment: 1 = litter received colostrum and post-colostrum milk feeding from their own dam; 2 = litter exchanged at birth to receive colostrum from a foster dam and then returned to their own dam for post-colostral milk feeding for the subsequent days; and 3 = litter exchanged at birth to receive colostrum and post-colostrum milk feeding from a foster dam, they remained with the foster dam for the subsequent days.

[2] Greatest standard error of mean (SEM).

[3] Trt = treatment; Day = 0 and 21.

Table 2.5 Association between bacteria genera present in colostrum, vaginal, and fecal samples of the sow and bacteria genera present in the gastrointestinal (GI) tract, fecal, and nasal samples of the young piglet

	Piglet			
Sow	GI Tract	Fecal	Nasal	
Colostrum	0.93806	0.71836	0.49668	r
	<0.0001	<0.0001	<0.0001	*P*
	0.88	0.51	0.24	R^2
Vaginal	0.99982	0.64664	0.42366	r
	<0.0001	<0.0001	<0.0001	*P*
	0.99	0.41	0.17	R^2
Fecal	0.95626	0.56564	0.34129	r
	<0.0001	<0.0001	<0.0001	*P*
	0.91	0.31	0.11	R^2

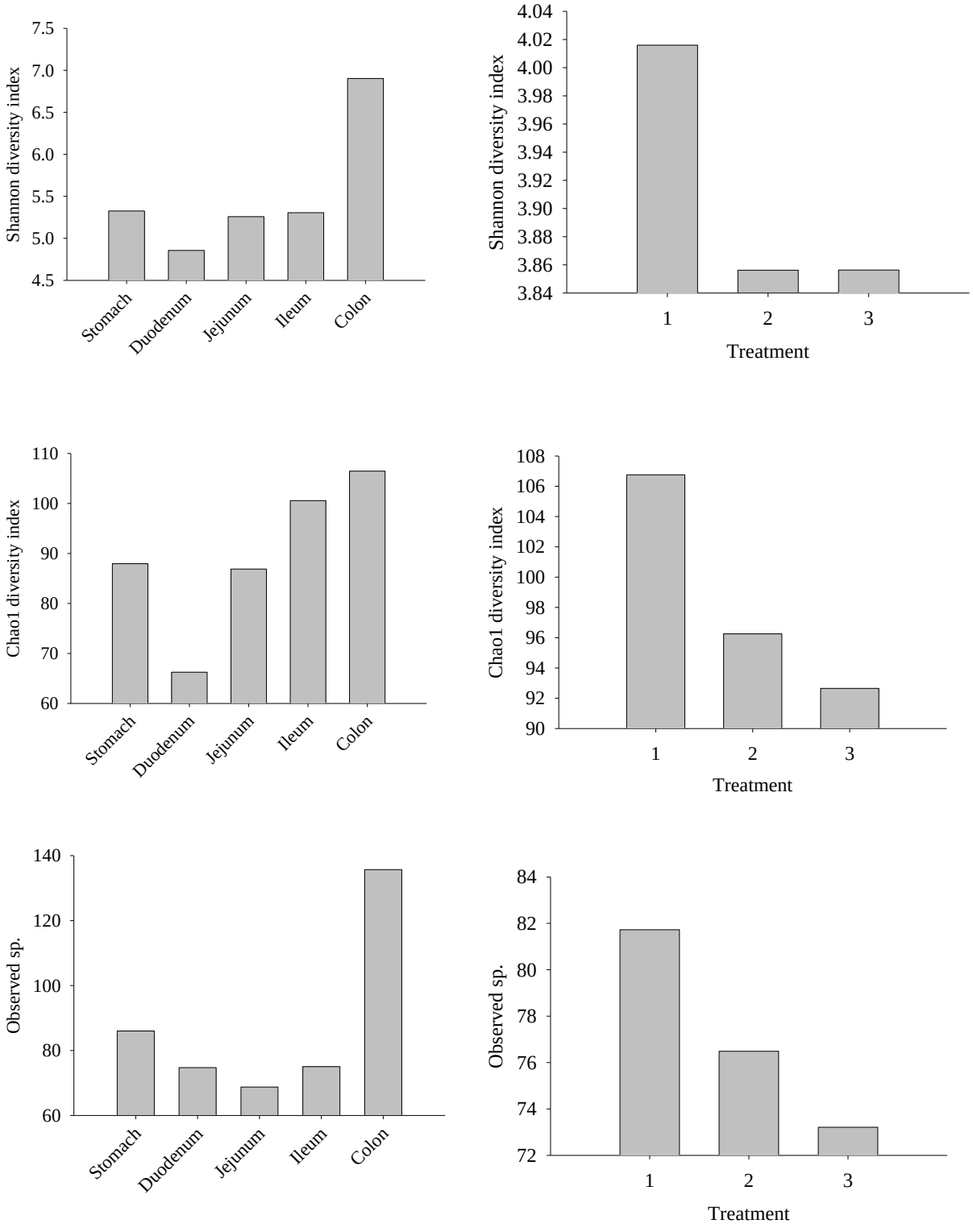

Figure 2.1. Microbial diversity estimates by gastrointestinal site and treatment.

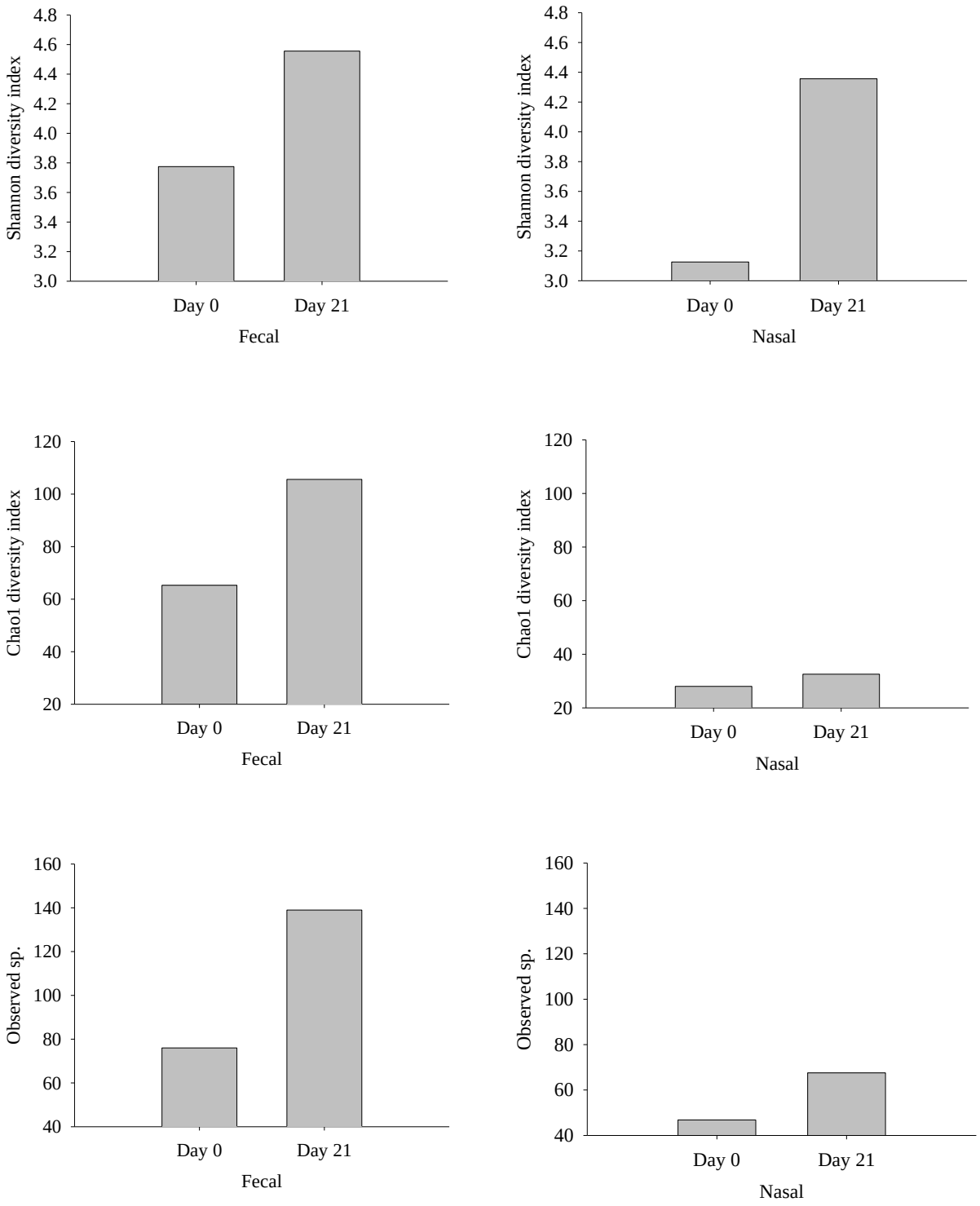

Figure 2.2. Microbial diversity estimates in fecal and nasal samples at day 0 and 21.

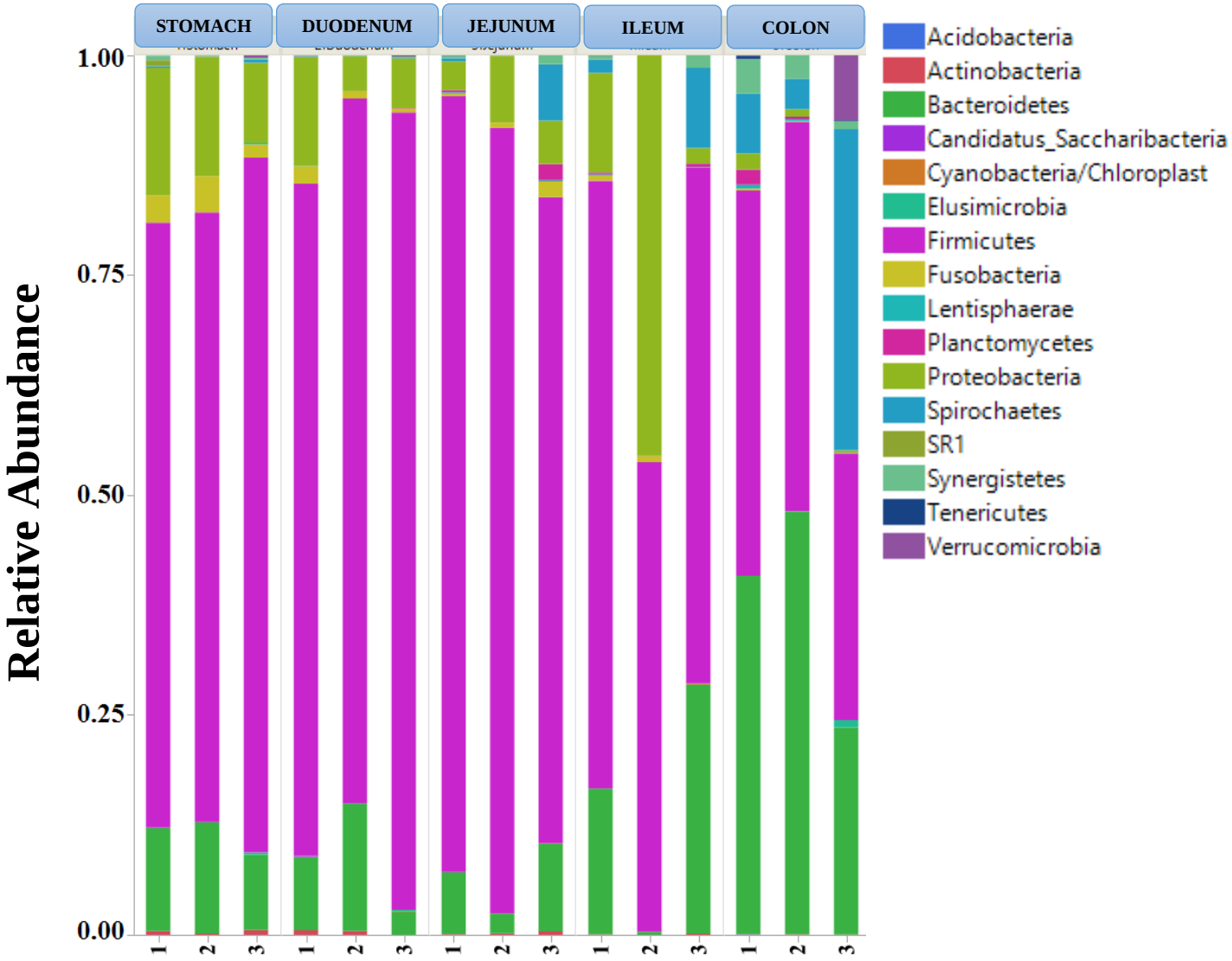

Figure 2.3. Bacterial phyla present in the gastrointestinal tract of the young pig by treatment.

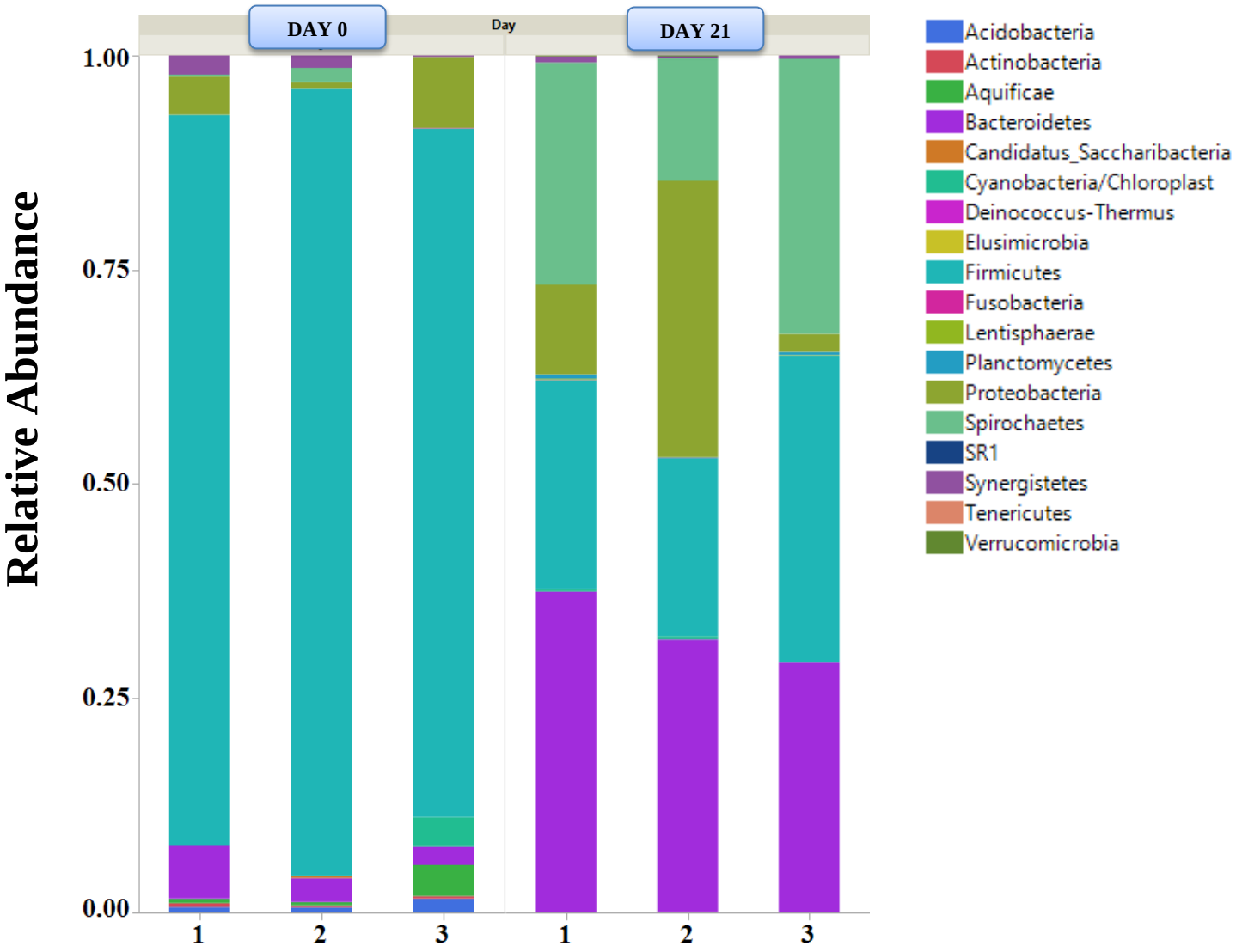

Figure 2.4. Bacterial phyla present in fecal samples at day 0 and 21 of the young pig by treatment.

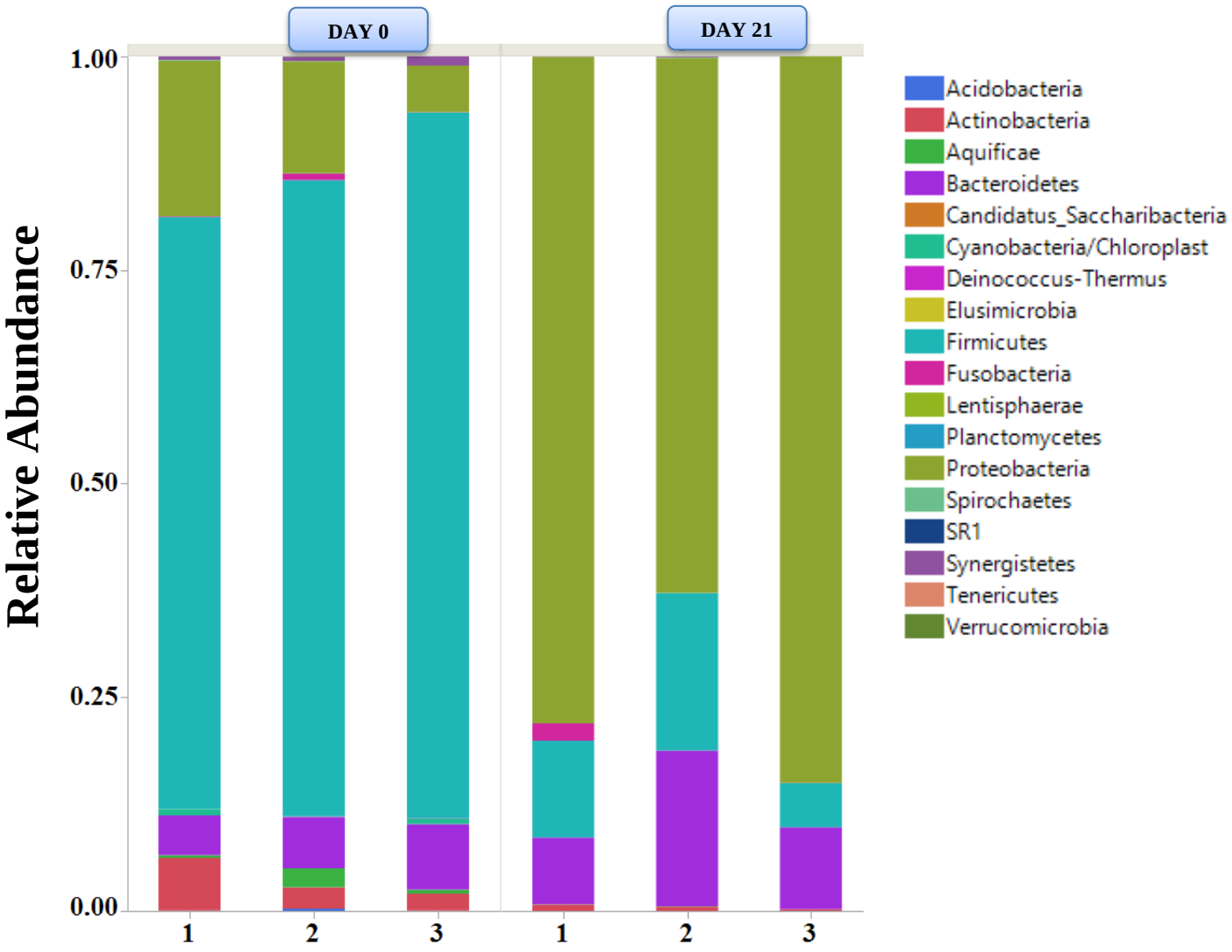

Figure 2.5. Bacterial phyla present in nasal samples at day 0 and 21 of the young pig by treatment.

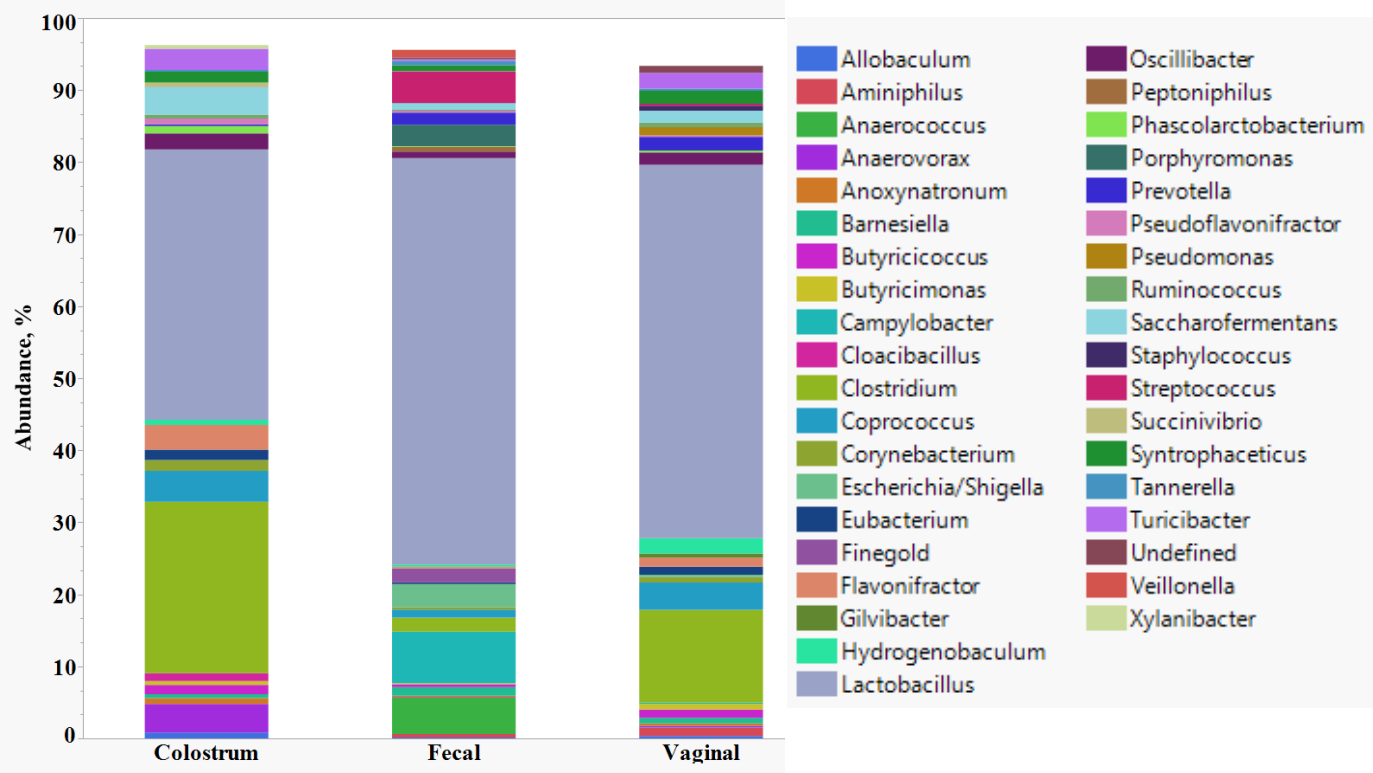

Figure 2.6. Bacterial genera present in colostral, fecal, and vaginal of the sow.

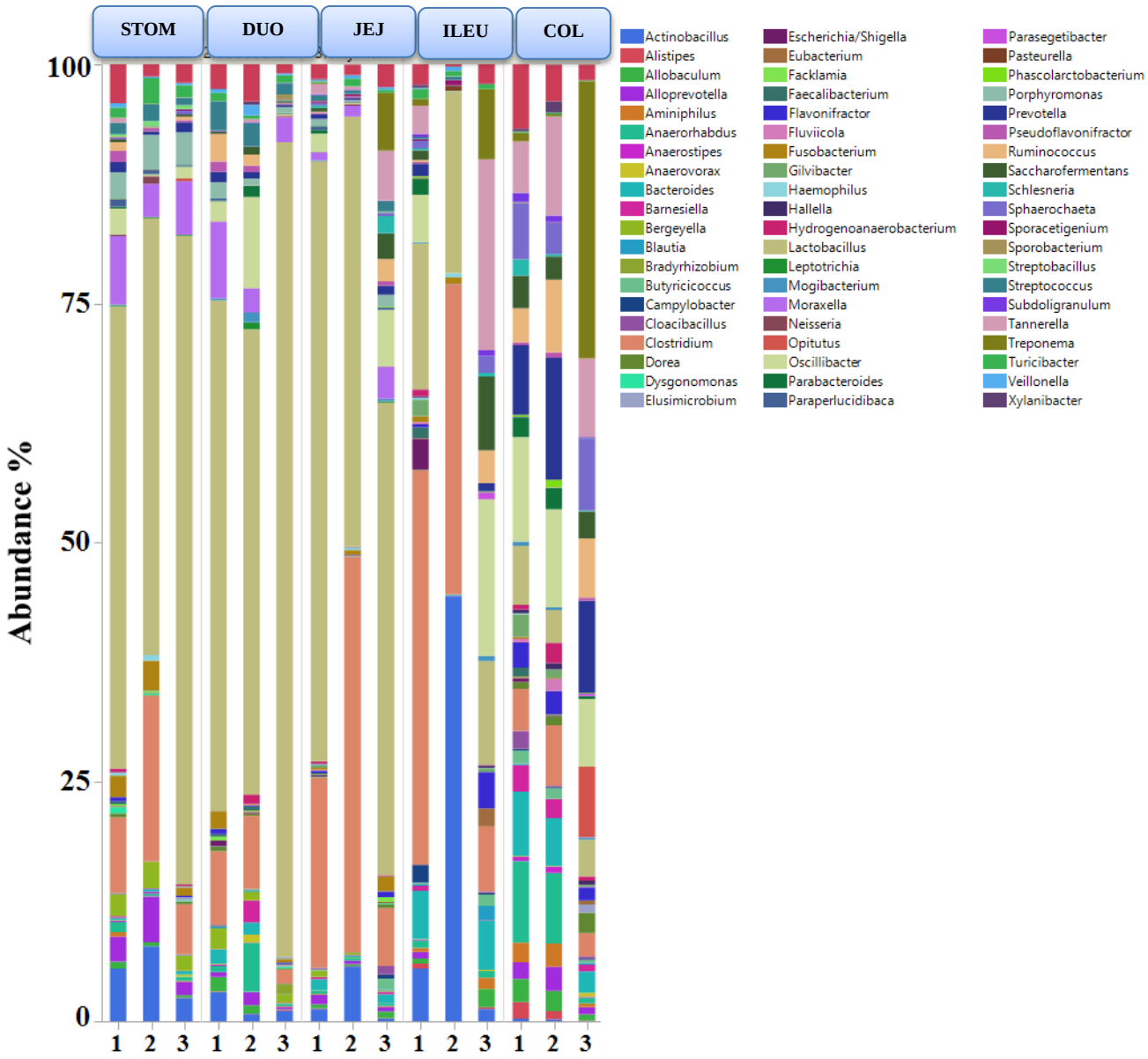

Figure 2.7. Bacterial genera present in the gastrointestinal tract of the young pig by treatment.

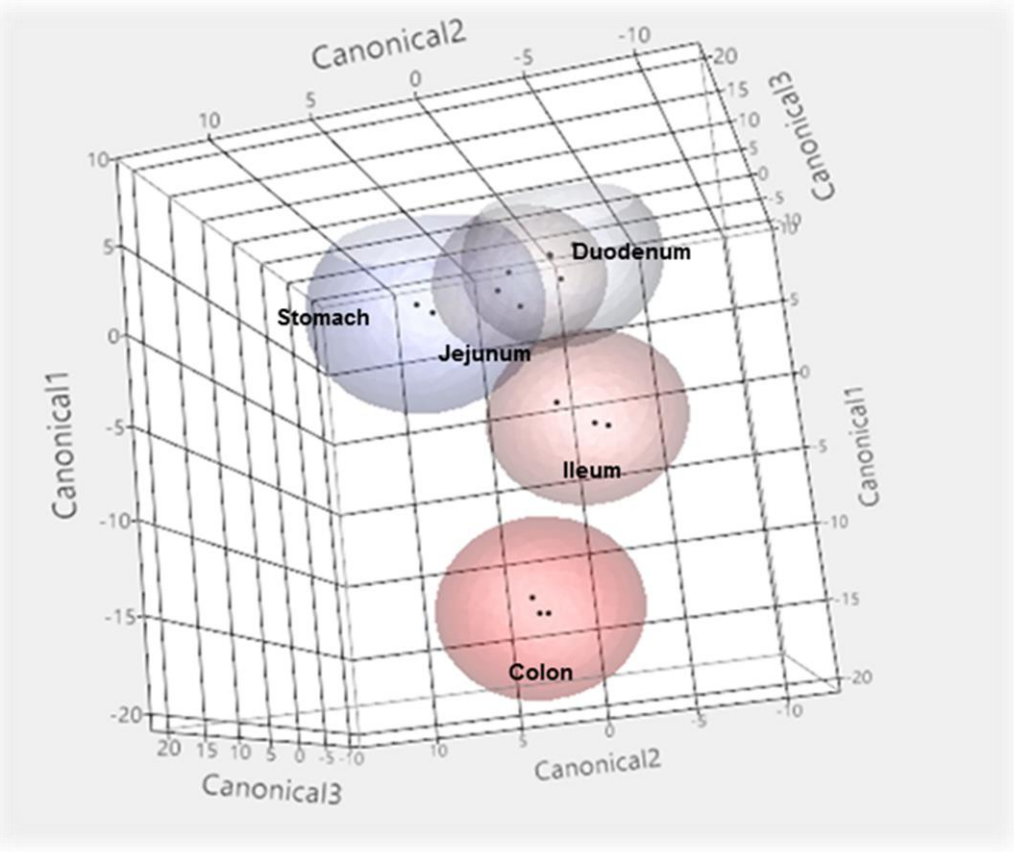

Figure 2.8. Discriminant analysis of bacterial genera present in the gastrointestinal tract of the young pig.

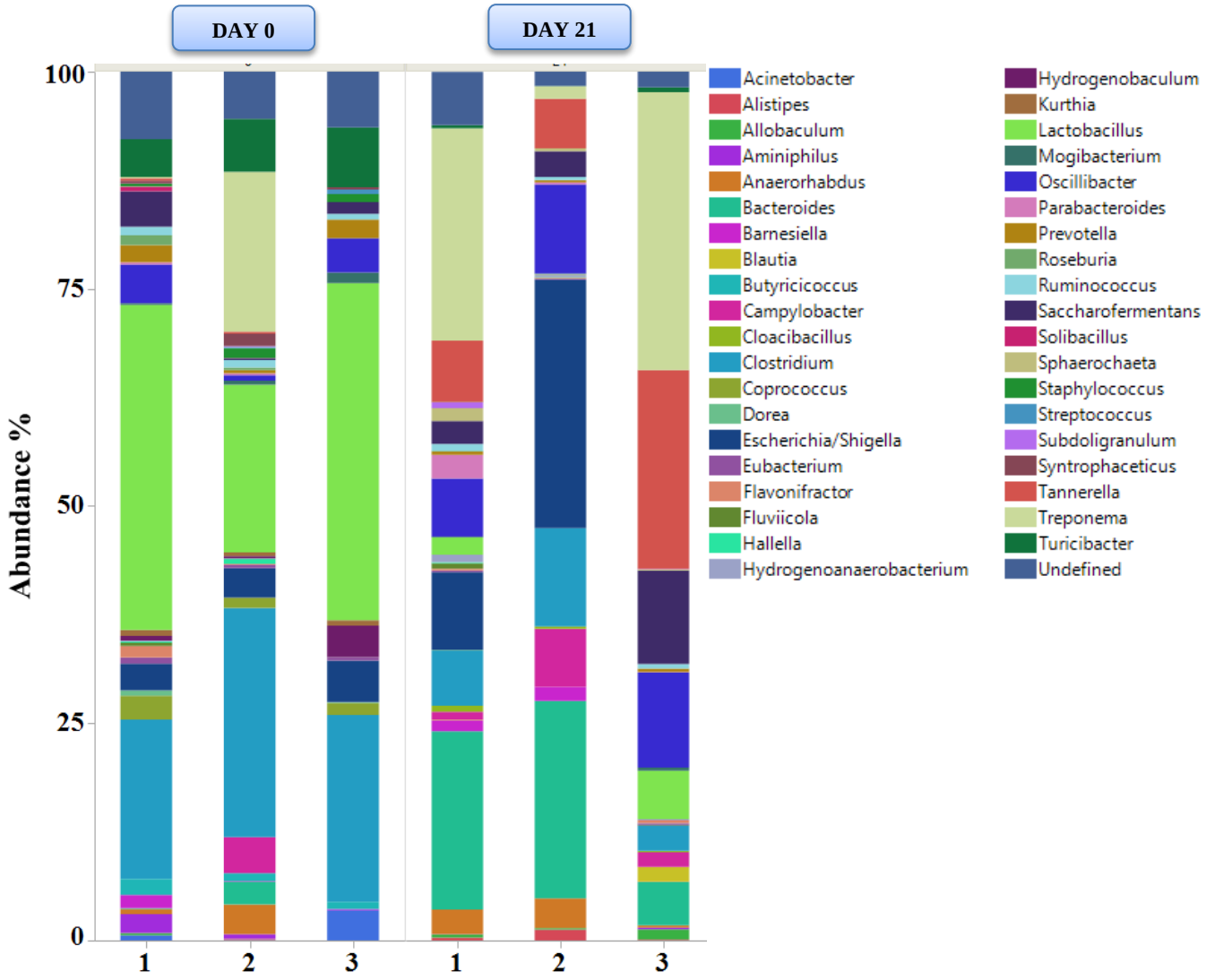

Figure 2.9. Bacterial genera present in fecal samples of the young pig by day and treatment

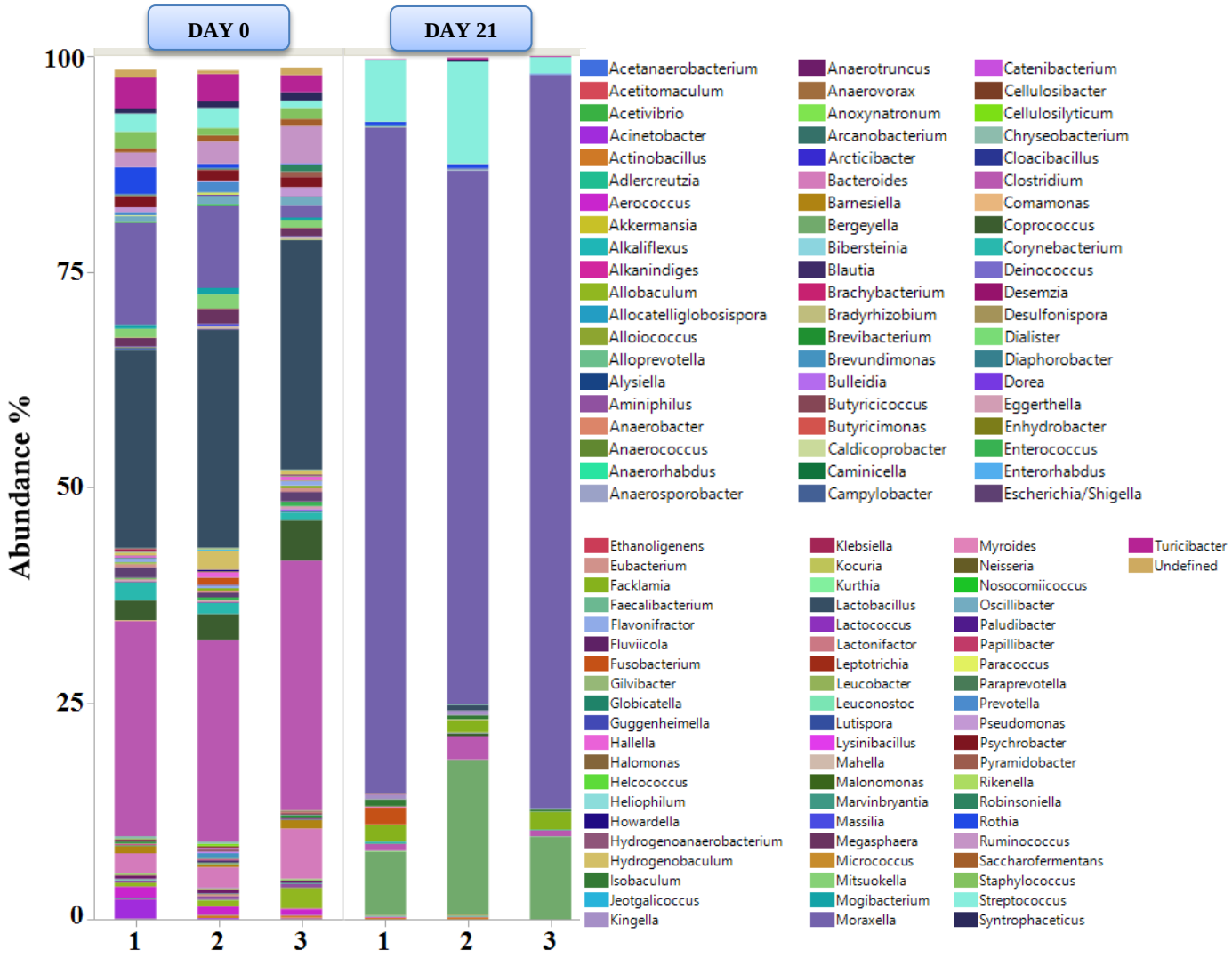

Figure 2.10. Bacterial genera present in nasal samples of the young pig by day and treatment