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Exposure to maternal feces in lactation influences piglet enteric microbiota, growth, and survival preweaning

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Abstract

It is known that gilt progeny performance is reduced compared with sow progeny. Previous research suggests that the presence of maternal feces in early life improves the health and survival of offspring. Therefore, we aimed to determine whether contact with feces from multiparous (MP) sows would improve the growth and survival of piglets born and reared on primiparous (P1) sows and if so, whether these differences are associated with the gut microbiota. Four treatments were applied for 10 days: Donor (n = 29) piglets had limited access to maternal feces as, each morning, sow feces were removed and placed in the crate of a P1 sow (P1-FT; n = 30 piglets) and P1-Con (n = 29) and MP-Con (n = 33) piglets had access to their own mothers' feces. All piglets were weighed on days 1, 3, 10, and 18. Fecal samples were collected from a subset of sows (n = 10/treatment) 3 days post farrow and from two female piglets/litter on days 10 and 18 (n = 20/treatment) and subject to 16S rRNA amplicon analysis. *Escherichia, Clostridium, Campylobacter,* and *Treponema* were more abundant in MP sows, while P1 sows had a higher abundance of *Lactobacillus* and *Prevotella*. At 10 days, P1 progeny fecal microbiota differed, and growth and survival were reduced when compared with MP progeny. No treatment effect was observed for P1-FT piglets ($P > 0.05$). Donor piglets had a different fecal microbiota and improved weight and survival then all other treatments ($P < 0.05$). Overall, the removal of sow feces from the farrowing crate improved piglet microbiota development, growth, and survival.

Key words: health, microbiota, parity, pig, postpartum, progeny

Introduction

The progeny of primiparous (P1) sows are born lighter and remain lighter throughout each phase of production and have a higher rate of mortality than multiparous (MP) sow progeny (Craig et al., 2017). Therefore, new methods for improving gilt progeny performance are needed. Recent research with humans and animals indicates that the gastrointestinal microbiota has a major role in health and survival (Nowland et al., 2019). To our knowledge, only one study investigating the differences in microbiota between P1 and MP sows has been published and it demonstrated a significant difference in fecal microbiota between P1 and MP sows within a UK herd (Gaukroger et al., 2021).

Abbreviations

DE	digestible energy
DNA	deoxyribonucleic acid
nMDS	nonmetric multidimensional scaling
OTU	operational taxonomic unit
rRNA	ribosomal ribonucleic acid

A preliminary study conducted by Aviles-Rosa et al. (2019) demonstrated that feed intake, growth, and white blood cell count were affected by whether the piglets had access to maternal feces during their first 7 days of life, or not, with those being exposed to maternal feces exhibiting improvements. However, since no investigation into the specifics surrounding the gastrointestinal microbiota were conducted, the cause of these differences remains to be determined.

Evidence suggests that piglets develop their gastrointestinal microbiota from contact with the sow and their environment and as piglets are housed exclusively with their sow in individual pens throughout lactation, it is likely that the sow drives this development. Therefore, the current study aimed to determine whether contact with feces from an older parity sow can improve growth and survival of piglets born and reared on primiparous sows and if so, whether these differences are associated with the gut microbiota. We hypothesized that 1) MP sows would have a fecal microbiota that is more diverse then P1 sows and 2) piglets born and reared on a P1 sow but exposed to feces from a MP sow would have an enteric microbiota similar to progeny of the older sows and would demonstrate improved growth and survival.

Materials and Methods

All procedures were conducted at the University of Adelaide Roseworthy piggery, South Australia, with the approval of the University of Adelaide's Animal Ethics Committee (AEC number: S-2019–053).

Animals and experimental procedures

A total of 121 Large White × Landrace primiparous (P1) and multiparous (MP) sows (parities 2–4; 2.95 ± 0.09) were employed in a series of 4 batches from September 2019 to January 2020. All sows were group housed and received 2.5 kg of commercial gestation diet daily (12.9 MJ DE/kg) throughout gestation. Sows were moved into a farrowing shed approximately 5 days before their expected farrow date and were housed in individual commercial farrowing crates (1.7 m × 2.4 m). The farrowing shed consisted of climate controlled and fully slatted plastic floored rooms. Upon entry into farrowing accommodation, sows received a commercial lactation diet (14.0 MJ DE/kg) at 2.5 kg/d until farrowing, thereafter the feeding level was gradually increased until it reached 7–8 kg by day 7 of lactation. All sows had ad libitum access to water. Two days before their due date, sows were induced to farrow by vulva injection of 100 µg cloprostenol at 8:00 a.m. and again at 2:00 p.m. Farrowing was monitored during staffed hours from 8:00 a.m. to 3:00 p.m. daily. Sows were allocated to one of four treatments on farrowing house entry:

- P1 control: maternal feces moved to each side at the rear of the pen to allow piglets easier access to feces (n = 29; P1-Con).
- P1 fecal transfer: maternal feces removed from the pen twice daily and a pooled fecal mixture from MP donor sows placed on each side at the rear of the pen to allow piglets easier access to feces (n = 30; P1-FT).
- MP control (parity 3 ± 0.7): maternal feces moved to each side at the rear of the pen to allow piglets easier access to feces (n = 33; MP-Con).
- MP donor (parities 3 ± 0.7): sow feces collected from the crate after feeding at 7:00 a.m. and 3:00 p.m. daily for placement in P1-FT pens. Therefore, these litters had reduced access to maternal feces (n = 29; Donor).

Treatments were imposed from farrowing for 10 days. The objective of the study was to assess the potential benefits to P1 progeny from exposure to MP sow feces due to the previously documented superior performance of MP sow progeny, and as such no treatment where MP sows received P1 sow feces were applied. Cross fostering was permitted within treatment at 24 h according to teat capacity (average litter size = 10.6 ± 1.2). All piglets within the litter were tagged with an individual identification number and weighed on days 1, 3, 10, and 18. Fecal samples were collected from a subset of sows 3 days post farrow (n = 10/treatment) and from two female focal piglets from each litter at 10 and 18 days of age (n = 20 piglets/treatment/timepoint). Sow fecal samples were collected by rectal stimulation with a gloved hand and direct collection into a sterile sample container. Piglet fecal samples were collected by isolating piglets in a sterile pen until defecation, whereby the feces were collected either directly from the rectum or off of the floor of the sterile container immediately after defecation. Once collected, fecal samples were placed on ice immediately, transported to a laboratory within 4 h, and stored at –80°C until required for microbial analysis. Sows and litters had no contact with antibiotics during lactation and the prior gestation. All piglet deaths were recorded. If a live-born piglet death occurred within the first 24 h of life, it was classified as pre-foster mortality and any that occurred after 24 h and prior to weaning, were classified as post-foster mortality. While total preweaning mortality was the sum of both pre- and post-foster deaths. Weaning occurred when piglets reached 18 days old.

Donor sample preparation and administration

All sows were fed at 7:00 a.m. and 2:00 p.m. daily to encourage defecation. Upon standing, all feces present in the Donor sows pen and any fresh fecal material was collected at 8:00 a.m. and 3:00 p.m. daily (n = 7–8 sows per batch). Once collected, the feces from all donor sows were immediately mixed in a bucket and evenly distributed to each P1-FT-treated pen where it was placed at the rear right and left corners of the pen to allow piglets to access easily. Approximately 2–4 kg of feces was administered per day. The quantity of feces administered to each pen differed daily as it depends on the amount of excreta present at the time of collection.

DNA extraction and 16S rRNA amplicon analysis

Total nucleic acid was extracted from freeze–dried piglet fecal samples by a modification of a South Australian Research and Development Institute (SARDI, Adelaide, Australia) proprietary method. Approximately 2 g of freeze–dried fecal sample was added to 20 mL extraction buffer (1.3 M guanidine thiocyanate, 1.5 M $NaCl_2$, 30 mM Tris-HCl, 65 mM phosphate buffer, 3.4% (w/v) sarkosyl, and 1.7% (w/v) polyvinylpolypyrrolidone) and

incubated for 1 h at 70°C prior to proceeding with the proprietary extraction method (Haling et al., 2011).

PCR amplification and sequencing of the V3-V4 region of the 16S rRNA gene was done by the Australian Genome Research Facility (AGRF). The V3-V4 region was PCR amplified over 29 cycles using forward primer 341-F (CCTAYGGGRBGCASCAG) and reverse primer 806-R (GGACTACNNGGGTATCTAAT). Amplicon sequencing was done on the illumina MiSeq platform (San Diego, CA) with 2 by 300 bp paired-end chemistry. Both positive and negative controls were used on every plate processed by AGRF. The positive control used was ZymoBIOMICS Microbial Community DNA Standard II (Log Distribution). The obtained reads are available under the accession number PRJNA682009 of the Sequence Read Archive of the National Centre for Biotechnology Information. Bioinformatic analysis of raw sequence data was done by the AGRF as follows. The paired-end sequences were assembled by aligning the forward and reverse reads using PEAR (Zhang et al., 2014; version 0.9.5) and the primers were identified and trimmed. All trimmed sequences were processed using Quantitative Insights into

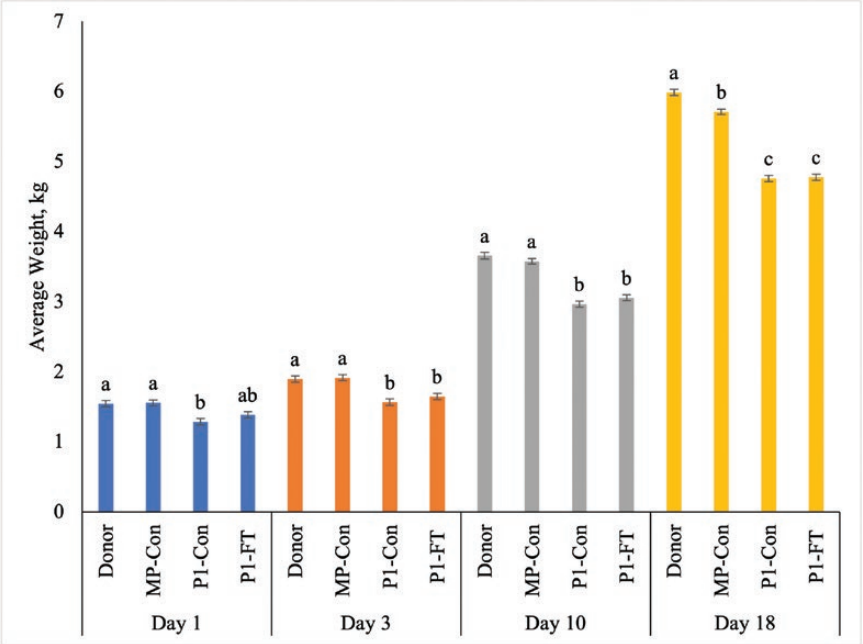

Figure 1. The effect of treatment (Donor, MP-Con, P1-Con, and P1-FT) on average piglet weight (kg ± SEM) at 1, 3, 10, and 18 days of age. Within age, means with differing letters are significantly different ($P < 0.05$).

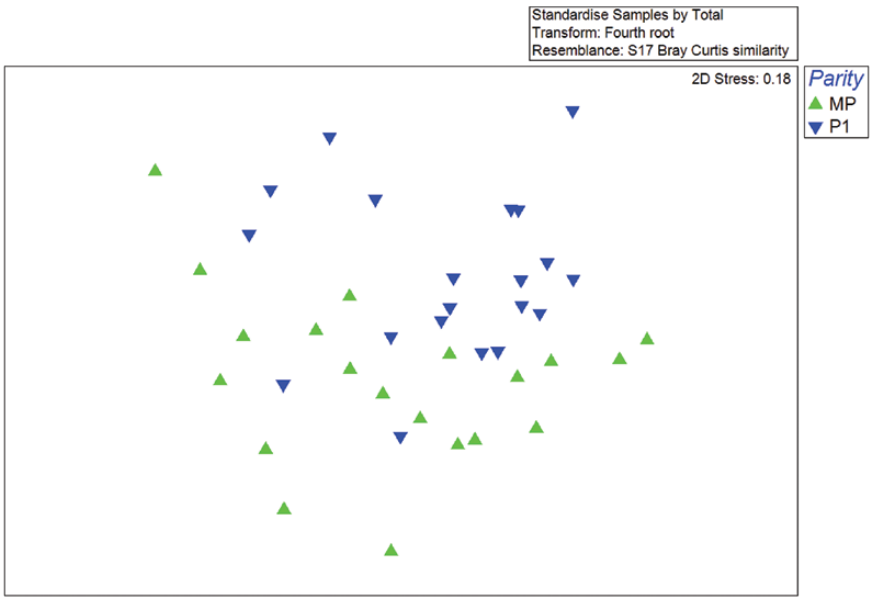

Figure 2. nMDS ordination showing the differences in relatedness of fecal bacterial genera from MP (triangle) or P1 (inverted triangle) sows, calculated using Bray–Curtis distances. Points on the ordination represent individual sow fecal samples which are positioned based on their similarity to other communities in a two-dimensional space. Points more closely clustered represent microbial communities more closely related to one another based on taxa composition and abundance.

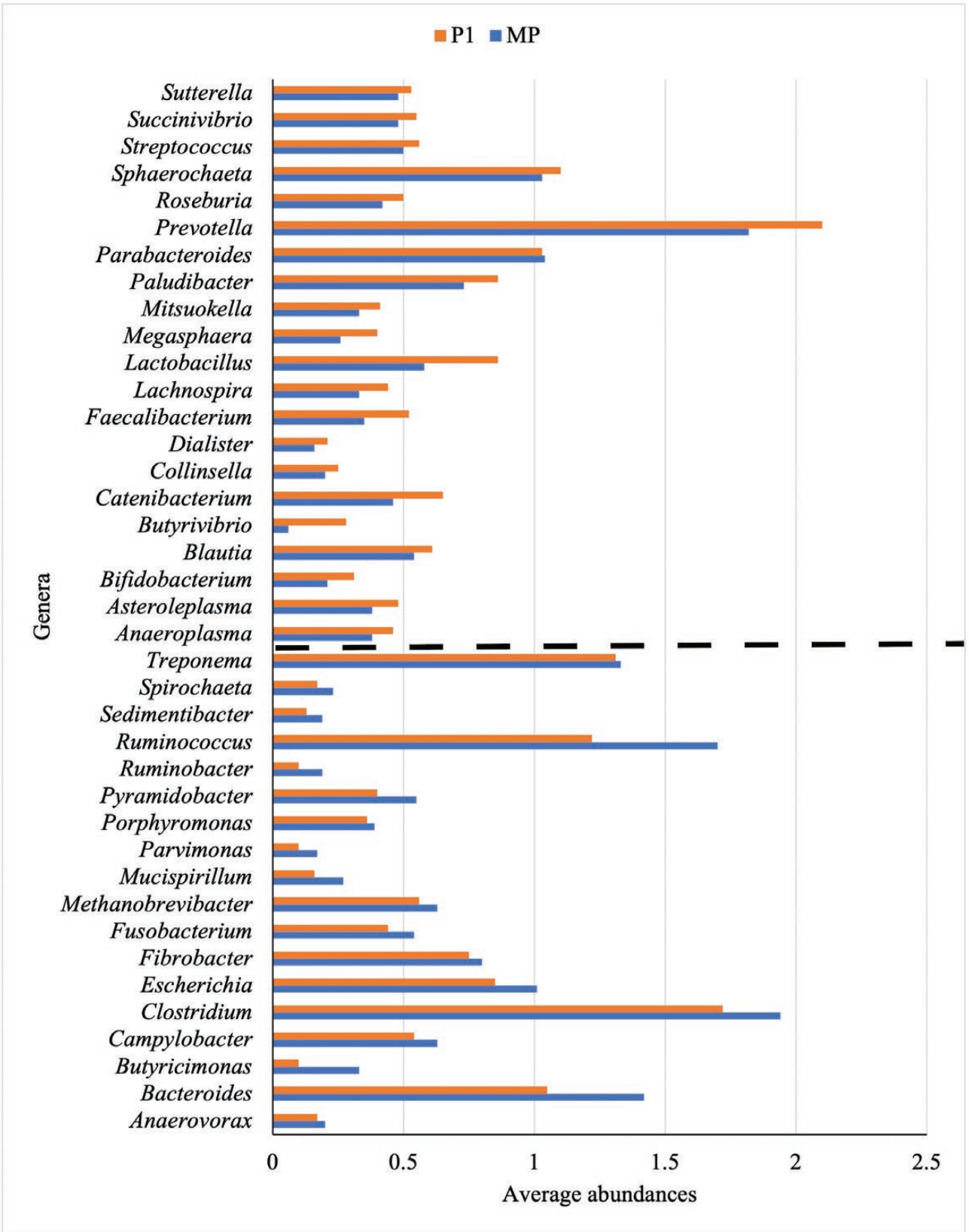

Figure 3. Average abundance of bacterial genera contributing significantly (average dissimilarity/standard deviation > 1) to the top 60% of dissimilarity between MP and P1 sows 3 days post-partum. Genera above the broken line were more abundant in P1 sows and all genera below the broken line were more abundant in MP sows.

Microbial Ecology (QIIME 1.8; Caporaso et al., 2010), USEARCH (version 8.0.1623; Edgar, 2010; Edgar et al., 2011), and UPARSE software (Edgar, 2013). Sequences were quality filtered, full length duplicate sequences were removed, and sorted by abundance. Singletons or unique reads in the dataset were discarded. Additionally, chimeric sequences were clustered and removed using "rdp_gold" database as the reference. Sequences were grouped into operational taxonomic units (OTUs) based on 97% sequence similarity. Using QIIME, taxonomy was assigned using the Greengenes database (Version 13.8, Aug 2013; DeSantis et al., 2006). All sequences corresponding to chloroplasts were removed.

Statistical analysis

All production data were tested for the normality of residuals and outliers before analysis. All weight data were analyzed using RStudio software (Version 1.1.456, Boston, MA). The "lmer" package was used to perform a general linear model to assess the effect of treatment on piglet weight. The fixed effects included in the model were treatment, sex, litter size weaned, age and treatment*age, with piglet ID specified as the random effect. However, sex and litter size weaned were not found to be significant so were removed from the final model. Data were expressed as estimated marginal means ± standard error of the mean (SEM), and a *P*-value threshold of 0.05. In SPSS v26 (IBM, USA), a generalized linear mixed model was fit to total preweaning mortality using a Poisson regression with treatment as a fixed effect and block as the random term.

The alpha diversity metrics, Shannon diversity (H′) index, Pielou's evenness (J′), and number of taxa (S), were calculated using DIVERSE (PRIMER6 PRIMER-E Ltd., Ivybridge, UK). Normality was tested within RStudio software (Version 1.1.456, Boston, MA) using the Shapiro–Wilk test. Those alpha diversity metrics that were found to be normally distributed were analyzed using an analysis of variance and those not normally distributed were analyzed using the Kruskal–Wallis test, with corrections for multiple tests using false discovery rate and a *P*-value threshold of 0.05.

Multivariate statistical techniques (PRIMER6, PRIMER-E Ltd., Ivybridge, UK) were used to analyze the fecal 16S rRNA bacterial taxonomic data. Similarities among fecal bacterial communities of sows and piglets from the 16S rRNA data metrics were analyzed using Bray–Curtis measures of similarity (Bray and Curtis, 1957), following standardization by sample total and fourth-root transformation. One-way analysis of similarity (ANOSIM; Clarke, 1993) on the Bray–Curtis similarity data was used to test if there were significant treatment and sow parity differences among fecal bacterial communities. If the global *R* statistic was significant ($P \leq 0.05$), then the significance of pairwise *R* statistics were investigated further. The *R* statistic value describes the extent of similarity among or between groups, with values close to unity (1) indicating that groups are entirely separate and a zero-value indicating that there is no difference among or between groups. In order to determine which individual bacterial taxa contributed most to the overall dissimilarity between statistically different groups, similarity percentages (SIMPER; Clarke, 1993) analyses were done and the overall average dissimilarity between sow or piglet fecal bacterial communities were calculated. The percentage contributions of significant taxa (average dissimilarity/standard deviation >1) to the top 60% of the average dissimilarities were calculated. Nonmetric multidimensional scaling (nMDS; Shepard, 1962; Kruskal, 1964) on Bray–Curtis similarity data was done to graphically illustrate relationships with parity.

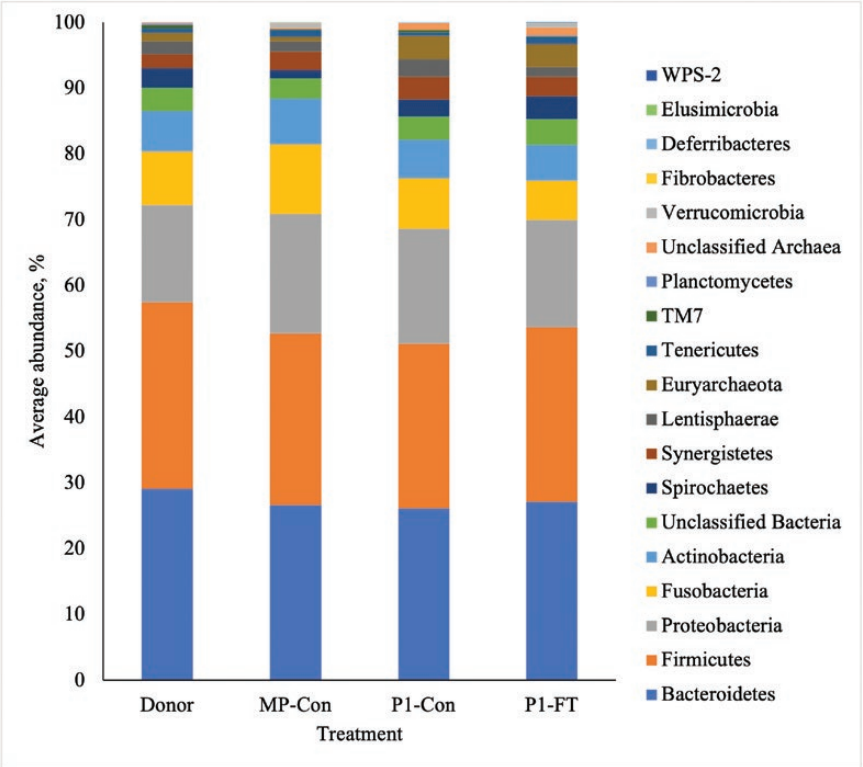

Figure 4. Average abundance of the phyla present within the feces of 10 day old piglets in the Donor, MP-Con, P1-Con, and P1-FT treatments.

Results

Performance

Significant treatment-related differences in piglet weight at 1, 3, 10, and 18 days of age were observed ($P < 0.001$; Figure 1). Consistently, Donor and MP-Con piglets were heavier than P1-Con and P1-FT piglets (Figure 1). Differences were small at day 1 but became larger with increasing age and by day 18 Donor piglets were heavier than piglets from all other treatments (Figure 1). There was a treatment effect on piglet preweaning mortality ($P = 0.008$). Piglets in the Donor treatment had a lower total preweaning mortality (0.89 ± 0.25 pigs per litter) than animals in the P1-Con (1.67 ± 0.30), P1-FT (1.82 ± 0.30), and MP-Con (1.41 ± 0.27) treatments.

The effect of parity on the sow's fecal microbiota

Across all 40 sow fecal samples, the total number of 16S rRNA sequenced reads were 2,458,821 with 1,869,533 reads retained after quality control, and an average of 46,738 16S rRNA sequenced reads per sow. Reads were clustered into 2,369 OTUs and assigned taxonomic classification.

For alpha diversity metrics, Shannon's diversity and the number of taxa, no significant differences were observed between parities ($P = 0.641$ and $P = 0.896$, respectively), while Pielou's evenness tended to be higher for P1 sows compared with MP sows ($P = 0.056$). Fecal bacterial genera differed between P1 and MP sows (ANOSIM, Global $R = 0.124$, $P = 0.004$) and is graphically demonstrated in Figure 2. At the genus level, the average dissimilarity between the fecal microbiota of P1 and MP sows was 23%. Of the taxa that could be classified to the genus level and were contributing significantly to the average dissimilarity between parity, those in the top 60% are displayed in Figure 3.

Treatment-related effects on the piglet's fecal microbiota

Across all 160 piglet fecal samples, the total number of 16S rRNA sequenced reads were 12,677,307 with 9,508,933 reads retained after quality control, and an average of 59,430 16S rRNA sequenced reads per piglet fecal sample. Reads clustered into 2,305 OTUs and assigned taxonomic classification.

Day 10

No genus level significant differences were observed between treatments for Shannon's diversity, Pielou's evenness, and the number of taxa ($P = 0.210$, $P = 0.419$, and $P = 0.539$, respectively). However, for beta diversity metrics, piglet fecal bacterial genera differed significantly with treatment (ANOSIM, Global $R = 0.112$, $P = 0.010$), with all pairwise comparisons being significantly different ($P < 0.010$), with the exception of P1-Con vs. P1-FT ($R = 0.007$, $P = 0.329$). The average abundance of phyla present within these piglet treatments are shown in Figure 4. The top six phyla in all treatments were Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, Actinobacteria, and Unclassified Bacteria, accounting for over 80% of the microbial community population (Figure 4). All treatments except P1-Con and P1-FT differed in the abundance of Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria, Unclassified Bacteria, Spirochaetes, Synergistetes, Lentisphaerae, Euryarchaeota, and Tenericutes. In addition to these differences, Donor piglets were significantly higher in Firmicutes than all other treatment groups and higher in TM7 when compared with P1-Con piglets, while Unclassified Archaea were higher in piglets reared on P1 sows (Figure 4).

Table 1. Genera contributing to the top 60% of dissimilarity of bacteria between Donor and MP-Con-treated 10-day old piglets as determined by SIMPER

	Donor	MP-Con	
Genera	Average abundance	Average abundance	Contribution %
Bacteroides	**1.64**	**2.05**	**2.01**
Escherichia	**1.17**	**1.47**	**1.77**
Unclassified	**0.61**	**0.77**	**1.76**
Rikenellaceae			
Clostridium	**1.81**	**1.85**	**1.59**
Lactobacillus	**1.18**	**1.24**	**1.47**
Campylobacter	**0.44**	**0.61**	**1.15**
Roseburia	**0.28**	**0.36**	**1.11**
Butyricimonas	**1.04**	**1.13**	**1.05**
Actinobacillus	**0.50**	**0.60**	**0.98**
Sutterella	**0.65**	**0.74**	**0.94**
Turicibacter	**0.28**	**0.38**	**0.94**
Eubacterium	**0.50**	**0.51**	**0.90**
Collinsella	**0.22**	**0.24**	**0.87**
Unclassified	**0.26**	**0.28**	**0.86**
Comamonadaceae			
Actinomyces	**0.39**	**0.41**	**0.83**
SMB53	**0.41**	**0.57**	**0.82**
Synergistes	**0.29**	**0.39**	**0.81**
Unclassified	**0.35**	**0.43**	**0.80**
Clostridiaceae			
Butyricicoccus	**0.44**	**0.48**	**0.70**
Streptococcus	**0.53**	**0.58**	**0.70**
Dialister	**0.10**	**0.26**	**0.71**
Prevotella	1.93	1.53	2.90
Unclassified S24-7	1.75	1.25	2.47
Unclassified	1.42	1.20	1.61
Bacteroidales			
Oscillospira	1.98	1.70	1.46
_CF231_	0.49	0.29	1.45
Fusobacterium	0.89	1.15	1.42
Unclassified	0.89	0.72	1.40
Clostridiales			
RFN20	0.72	0.62	1.40
_p-75-a5_	0.73	0.62	1.21
Sphaerochaeta	0.38	0.26	1.13
Odoribacter	0.38	0.31	0.97
Unclassified	1.44	1.42	0.94
Ruminococcaceae			
Unclassified	0.61	0.55	0.94
Christensenellaceae			
Parabacteroides	1.13	1.09	0.93
Unclassified	0.46	0.45	0.90
Bacteria			
Unclassified	0.38	0.29	0.89
GMD14H09			
Unclassified	0.97	0.8	0.85
Lachnospiraceae			
Ruminococcus	1.19	1.08	0.85
Flexispira	0.25	0.16	0.78
Bulleidia	0.26	0.16	0.76
vadinCA11	0.20	0.19	0.73
Dorea	0.75	0.62	0.72
Unclassified	0.22	0.17	0.72
Paraprevotellaceae			
Unclassified	0.68	0.52	0.72
Mogibacteriaceae			
Unclassified	0.24	0.16	0.70
Firmicutes			
Blautia	0.54	0.44	0.70

Bold depicts those genera that have a higher abundance in MP-Con-treated piglets. Overall, average dissimilarity between treatments was 34%.

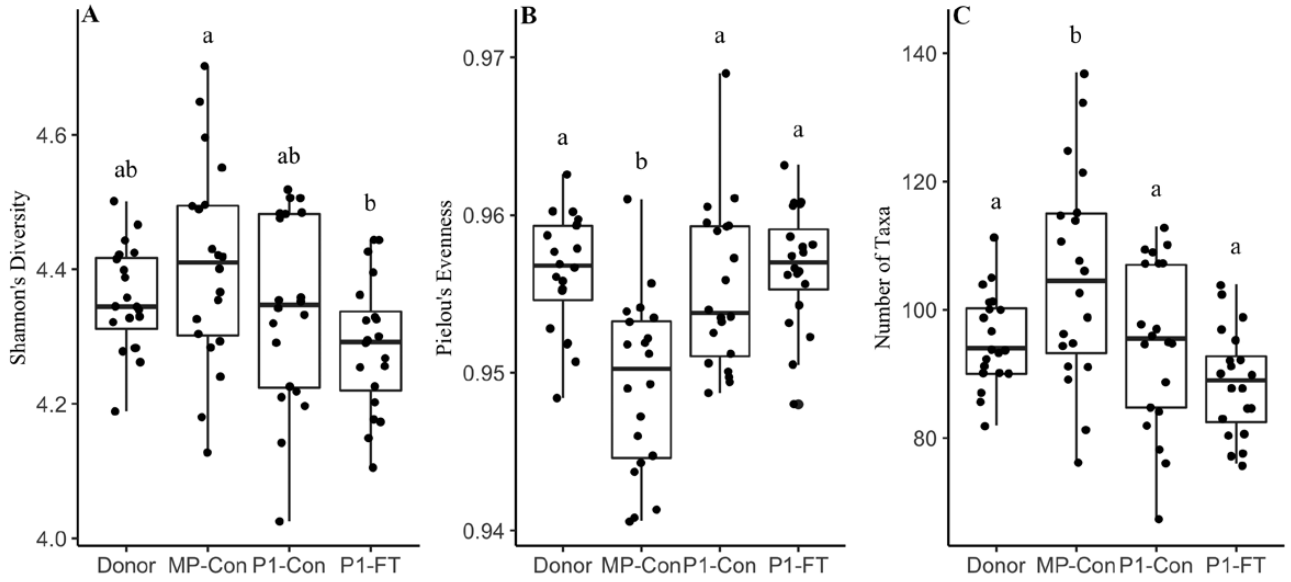

Figure 5. Boxplots demonstrating the differences between bacterial genera for piglets in treatments: Donor, MP-Con, P1-Con, and P1-FT for (A) Shannon's diversity, (B) Pielou's Evenness, and (C) Number of taxa. Subscripts without a common letter denote a significant difference between treatments ($P < 0.05$).

The top 60% of genera driving the differences observed between Donor vs. MP-Con piglets as determined by SIMPER analysis are listed in Table 1. The mean relative abundance of multiple genera differed between 10-day old, P1-Con, and Donor piglets and had an average dissimilarity of 35%. Of those that were in the top 60% and could be classified to the genus level, *Bacteroides*, *Prevotella*, *Butyricimonas*, *Lactobacillus*, *Odoribacter*, *Blautia*, *Clostridium*, *Peptostreptococcus*, *Actinomyces*, *Sutterella*, *Phascolarctobacterium*, *Dorea*, and *Flexispira* were more abundant in Donor piglets, while *Escherichia*, *Turicibacter*, *Roseburia*, *Sphaerochaeta*, *Synergistes*, *Parabacteroides*, *Campylobacter*, *Enterococcus*, *Eubacterium*, *Actinobacillus*, *Bulleidia*, *Ruminococcus*, and *Butyricicoccus* were more abundant in P1-Con piglets. Furthermore, when assessing the difference between P1-Con and MP-Con piglets, the average dissimilarity of bacteria was 37% and the differences observed were similar to those differences observed between P1-Con and Donor piglets with exception to, *Fusobacterium*, *Oscillospira*, *Turicibacter*, *Roseburia*, *Clostridium*, *Campylobacter*, *Dialister*, and *Butyricicoccus* being more abundant in MP-Con animals and *Blautia*, *Collinsella*, and *Dorea* being more abundant in P1-Con-treated animals.

There was an average dissimilarity of 33% between the fecal microbiota of 10-day old piglets in the Donor and P1-FT treatments at genus level. When assessing the top 60% of genera, *Fusobacterium*, *Oscillospira*, *Lactobacillus*, *Odoribacter*, *Butyricimonas*, *Actinomyces*, *Sutterella*, *Ruminococcus*, *Parabacteroides*, *Blautia*, *Eubacterium*, *Dorea*, and *Anaerotruncus*, were more abundant in Donor piglets, while *Bacteroides*, *Prevotella*, *Escherichia*, *Campylobacter*, *Roseburia*, *Sphaerochaeta*, *Peptostreptococcus*, *Flexispira*, *Actinobacillus*, *Turicibacter*, *Paludibacter*, *Faecalibacterium*, *Synergistes*, *Bulleidia*, and *Mogibacterium* were more abundant in P1-FT piglets. The differences observed were similar to those observed between P1-FT and MP-Con piglets, with exception to *Bacteroides*, *Actinobacillus*, *Collinsella*, *Dialister*, and *Streptococcus* being more abundant in MP-Con animals and *Oscillospira*, *RFN20*, and *Blautia* being more abundant in P1-FT animals. The average dissimilarity between the fecal microbiota of piglets at 10 days of age for MP-Con and P1-FT was 36%.

Day 18

At day 18 alpha diversity metric, Shannon's diversity differed with MP-Con piglets having a higher diversity than P1-FT piglets ($P = 0.024$; Figure 5), while all other comparisons were not significantly different. Bacterial community evenness and the number of taxa also differed, with MP-Con piglets having a lower evenness but a higher number of taxa than all other treatments ($P < 0.001$; Figure 5). Treatment-associated differences for beta diversity metrics were observed in 18-day old piglet fecal bacterial genera (ANOSIM, Global $R = 0.041$, $P = 0.016$). The significant pairwise differences observed were between the Donor vs. P1-FT ($R = 0.082$, $P = 0.015$) and MP-Con vs. P1-FT ($R = 0.112$, $P = 0.005$). The top 60% of genera contributing significantly to the difference between 18-day old piglets within the P1-FT and Donor treatment groups are shown in Table 2.

Differences also existed between piglets in the P1-FT and MP-Con treatment groups, the top 60% of the differences in genera are shown in Table 3.

Discussion

As the lactation environment involves the housing of piglets in a pen exclusively with one sow, the sow's feces will influence the developing intestinal microbiota within her piglets via coprophagy, which has been documented as a natural phenomenon in pigs (Aviles-Rosa et al., 2019). Additionally, sow parity differences have been noted for their piglet's nasal mucosal bacterial colonization (Brean et al., 2016), so an expectation of sow parity differences on piglet's enteric bacterial colonization is reasonable. In our study, MP and P1 sows had significantly different fecal microbiota 3 days postpartum, with the differences observed presented in the feces of their piglets at day 10 of lactation. These data are similar to the findings of Gaukroger et al. (2021) who demonstrated differences between MP and P1 sow fecal microbiota both prior to and post farrowing. Additionally, similar to previous literature, the present study observed significantly lower growth and survival throughout lactation in P1 progeny when compared with MP sow progeny (Carney-Hinkle et al., 2013; Craig et al., 2017). The

Table 2. Genera contributing to the top 60% of dissimilarity between Donor and P1-FT-treated 18-day old piglets as determined by SIMPER

Genera	Donor	P1-FT	
	Average abundance	Average abundance	Contribution %
Escherichia	**1.29**	**1.40**	**1.95**
Prevotella	**1.19**	**1.47**	**1.85**
Bacteroides	**1.26**	**1.42**	**1.56**
Unclassified	**1.17**	**1.22**	**1.56**
Christensenellaceae			
_p-75-a5_	**1.05**	**1.15**	**1.53**
Unclassified S24-7	**1.66**	**1.67**	**1.34**
Prevotella	**0.61**	**0.72**	**1.13**
Campylobacter	**0.48**	**0.65**	**1.08**
Ruminococcus	**1.09**	**0.97**	**1.05**
_CF231_	**0.62**	**0.65**	**1.00**
Roseburia	**0.48**	**0.42**	**0.99**
Clostridium	**0.91**	**0.92**	**0.98**
Unclassified	**0.98**	**1.09**	**0.96**
Lachnospiraceae			
Anaerovibrio	**0.18**	**0.32**	**0.90**
Synergistes	**0.56**	**0.58**	**0.85**
Streptococcus	**0.50**	**0.56**	**0.79**
Faecalibacterium	**0.38**	**0.42**	**0.77**
Unclassified	**0.24**	**0.36**	**0.75**
Erysipelotrichaceae			
Turicibacter	**0.27**	**0.29**	**0.74**
Unclassified	**0.21**	**0.31**	**0.73**
Peptostreptococcaceae			
Butyricimonas	**0.80**	**0.87**	**0.71**
Dialister	**0.14**	**0.21**	**0.67**
Unclassified	1.33	1.18	1.63
Clostridiales			
Unclassified	1.51	1.25	1.29
Bacteroidales			
Unclassified	0.44	0.41	1.23
Rikenellaceae			
Unclassified	0.52	0.39	1.19
p-2534-18B5			
Paludibacter	0.41	0.19	1.17
Lactobacillus	1.11	0.98	1.07
Dorea	0.62	0.51	1.06
Unclassified	1.71	1.53	1.02
Ruminococcaceae			
Clostridium	0.85	0.61	0.98
Ruminococcus	0.90	0.87	0.98
Sphaerochaeta	0.62	0.54	0.94
Oscillospira	1.99	1.85	0.92
Blautia	0.73	0.70	0.90
Collinsella	0.42	0.22	0.88
Unclassified RF39	0.55	0.46	0.88
Megasphaera	0.35	0.31	0.86
Treponema	0.51	0.33	0.86
_L7A_E11_	0.34	0.23	0.83
Catenibacterium	0.33	0.23	0.83
Odoribacter	0.35	0.19	0.82
Flexispira	0.47	0.46	0.82
Unclassified	1.24	1.12	0.82
Clostridiales			
Sutterella	0.59	0.47	0.82
Parabacteroides	1.15	1.10	0.80
RFN20	0.69	0.62	0.79
Acidaminococcus	0.24	0.21	0.78
Unclassified	0.34	0.32	0.78
GMD14H09			
Pyramidobacter	0.29	0.22	0.75
Peptococcus	0.34	0.18	0.69
Unclassified	0.36	0.32	0.69
Firmicutes			
Eubacterium	0.68	0.58	0.68
Unclassified	0.34	0.24	0.68
Victivallaceae			
Unclassified	0.52	0.49	0.67
Lachnospiraceae			
Actinobacillus	0.47	0.54	0.66

Bold depicts those species that have a higher abundance in P1-FT-treated piglets. Overall, average dissimilarity between treatments was 31%.

production improvements in MP sow progeny in the present study are interesting as MP sows had a higher abundance of potentially pathogenic bacteria such as *Escherichia*, *Clostridium*, *Campylobacter*, and *Treponema* compared with P1 sows, while P1 sows had a higher abundance of beneficial bacteria, *Lactobacillus* and *Prevotella*, with these same differences observed in the progeny of MP and P1-Con animals at 10 days of age. It is likely that passive immunity transferred from sows to their piglets could compensate for these differences. In addition, previous work has shown that gilt progeny have a number of anatomical differences indicative of delayed development that persist to weaning when compared with sow progeny (Craig et al., 2019), and in some cases MP sows have increased IgG and IgA concentrations in serum and milk/colostrum (Carney-Hinkle et al., 2013). Therefore, given the fact that we were able to demonstrate differences in microbiota, it may be more complex than originally thought and it is likely that it is a combination of these differences that collectively contribute to the parity differences observed in piglet performance.

The higher abundance of potentially pathogenic bacteria observed within MP sow feces and piglets within the MP-Con treatment may also provide some insight as to why the piglets in the Donor treatment performed better than all other treatment groups. Their improved growth performance is possibly due to them having limited exposure to the potentially pathogenic bacteria within the sow's feces for the first 10 days of life, arguably during the time of the highest risk of disease for the piglet (Lay et al., 2015). The reduction in preweaning mortality in these pigs further supports this suggestion. Our findings contrast those of Aviles-Rosa et al. (2019), who documented poorer performance for pigs deprived of maternal feces. Although Aviles-Rosa et al. (2019) recorded weight throughout lactation, no treatment effects were seen in weight until 56 days postweaning, while we only measured growth to 18 days. In contrast, studies comparing flooring type observed similar findings to the present study and demonstrated the positive effects of crate cleanliness on production outcomes (Mabry et al., 1982; Rantzer and Svendsen, 2001).

That the addition of MP feces to the pen of P1 piglets provided no evident advantage or disadvantage to the piglets is intriguing. This implies that either sow feces do not impact piglet performance or that, in our study, the piglets had inadequate contact with minimal coprophagy. It is also possible that the quantity of feces added to the pen was not sufficient,

Table 3. Genera contributing to the top 60% of dissimilarity between MP-Con and P1-FT-treated 18-day old piglets as determined by SIMPER

Genera	MP-Con Average abundance	P1-FT Average abundance	Contribution %
Prevotella	**1.51**	**2.19**	**3.30**
Escherichia	**1.32**	**1.40**	**1.76**
Unclassified S24-7	**1.36**	**1.67**	**1.67**
p-75-a5	**1.13**	**1.15**	**1.62**
CF231	**0.56**	**0.65**	**1.09**
Roseburia	**0.37**	**0.42**	**1.00**
Campylobacter	**0.57**	**0.65**	**0.93**
Flexispira	**0.34**	**0.46**	**0.92**
Megasphaera	**0.21**	**0.31**	**0.89**
Anaerovibrio	**0.13**	**0.32**	**0.84**
Unclassified RF39	**0.46**	**0.46**	**0.78**
Unclassified GMD14H09	**0.29**	**0.32**	**0.74**
Streptococcus	**0.50**	**0.56**	**0.73**
RFN20	**0.53**	**0.62**	**0.73**
Unclassified Paraprevotellaceae	**0.34**	**0.47**	**0.72**
Treponema	**0.27**	**0.33**	**0.72**
Unclassified Peptostreptococcaceae	**0.25**	**0.31**	**0.71**
Peptostreptococcus	**0.25**	**0.25**	**0.68**
Unclassified Erysipelotrichaceae	**0.30**	**0.36**	**0.68**
Dialister	**0.15**	**0.21**	**0.63**
Unclassified Christensenellaceae	1.26	1.22	1.68
Bacteroides	1.62	1.42	1.57
Unclassified Clostridiales	1.25	1.18	1.50
Unclassified Rikenellaceae	0.51	0.41	1.20
Enterococcus	0.34	0.27	1.14
Unclassified p-2534-18B5	0.47	0.39	1.13
Unclassified Bacteroidales	1.37	1.25	1.10
Synergistes	0.71	0.58	1.09
Dorea	0.71	0.51	1.05
Ruminococcus	1.99	1.84	2.03
Clostridium	0.90	0.61	1.00
Oscillospira	1.94	1.85	0.99
Blautia	0.72	0.70	0.91
Unclassified Coriobacteriaceae	0.36	0.30	0.91
Unclassified Ruminococcaceae	1.60	1.53	0.90
Unclassified Lachnospiraceae	1.10	1.09	0.89
Lactobacillus	1.03	0.98	0.89
Clostridium	0.99	0.92	0.86
Sphaerochaeta	0.55	0.54	0.85
Parabacteroides	1.20	1.10	0.84
Faecalibacterium	0.44	0.42	0.83
Unclassified Clostridiales	1.27	1.12	0.80
Catenibacterium	0.29	0.23	0.80
Unclassified Clostridiaceae	0.25	0.12	0.80
Turicibacter	0.43	0.29	0.78
Paludibacter	0.22	0.19	0.75
Sutterella	0.56	0.47	0.75
Unclassified Comamonadaceae	0.64	0.58	0.73
Butyricimonas	0.92	0.87	0.73
Collinsella	0.32	0.22	0.72
Odoribacter	0.29	0.19	0.72
L7A_E11	0.27	0.23	0.71
Actinomyces	0.34	0.19	0.70
Moryella	0.31	0.16	0.70
Eubacterium	0.63	0.58	0.66
Methanobrevibacter	0.35	0.21	0.64
Pyramidobacter	0.24	0.22	0.63

Bold depicts those species that have a higher abundance in P1-FT-treated piglets. Overall, average dissimilarity between treatments was 32%.

especially early in lactation when sows did not defecate often so the amount of fresh feces to deliver was sometimes limited. To ensure that feces were present within the pen at birth, the fecal transfers started prior to the onset of parturition. It is possible that the freshness of the feces at the time of birth could have influenced this. Additionally, it is possible that the amount of time the piglets spent interacting with the feces could have had an influence and since piglets can differentiate their sow's feces (Horrell and Hodgson, 1992), donor sow feces may not have been as attractive as their own mothers would have been.

In the present study, MP and P1 progeny maintained production differences throughout lactation, and previous studies demonstrate that these deficits remain beyond weaning (Craig et al., 2017). The parity-specific differences observed in the piglet fecal microbiota at day 10 were not as evident by day 18 as control animals did not differ (MP-Con and P1-Con). Previous studies by our research group and others have documented this age-related change in fecal microbiota of piglets during lactation (Gaukroger et al., 2020; Nowland et al., 2020a, 2020b). However, to our knowledge, no analysis of fecal microbiota between piglets reared on different parity sows have been documented. Diet and environment shape the developing intestinal microbiota of the neonate (Nowland et al., 2019). Therefore, it is likely that the sow's microbiota has a greater influence on development of the piglet's microbiota early in lactation but, as the piglets age and are exposed to more environmental stimuli (handling by stock people, eating the sows feed, etc.), the impact of the sow diminishes.

Conclusion

To our knowledge, this is the first study to characterize and compare the fecal microbiota of different parity sows with their piglets, and to document how the addition of MP sow feces to the pen of P1 sows influences piglet development. The identification of parity-specific microbial differences throughout lactation may allow for the development of easy to implement on-farm approaches to improve gut health and performance of the sow during lactation and in turn influence piglet's growth and survival. The present results suggest that MP and P1 sows

do have a significantly different fecal microbiota that influences the piglet fecal microbiota until at least 10 days of age. As other studies have also demonstrated, the growth and survival of P1 sow progeny was significantly reduced preweaning when compared with MP sow progeny, however it is uncertain as to whether differences in microbiota cause these production differences. It is evident that the inclusion of MP feces to the pen of a P1 sow provided no benefit or hinderance to the piglets reared in that environment. However, the removal of feces from the pen for the first 10 days significantly improved piglet weight and survival to weaning. Further investigation into the possibility of altering the sow's fecal microbiota through dietary manipulation to positively influence the piglet's microbiota and growth are needed.

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Conflict of interest statement

The authors declare that they have no conflicts of interests.

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