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Effect of Dam Parity on Progeny Gastrointestinal Microbiota

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Effect of Dam Parity on Progeny Gastrointestinal Microbiota

The gastrointestinal microbiota of neonatal pigs may be affected by dam parity.

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Summary

Litter performance, progeny growth performance, and progeny health status may be affected by dam parity. The objective of the current experiment was to evaluate gastrointestinal microflora, as a measure of gut health, in progeny derived from first parity (P1) compared to fourth parity (P4) dams. Fecal samples were collected from the progeny (n = 6 pigs/litter) of P1 and P4 dams (n = 4 from each parity, P1 and P4) on days 1, 7, and 14 following parturition. Denaturing gradient gel electrophoresis was utilized to characterize gastrointestinal microbial populations and to calculate similarity and diversity indices. The similarity index represents the percentage of the microbial population that is similar within a group (P1 vs. P4). Diversity indices (Shannon's and Simpson's) represent the differences of the bacterial species within the microbial population. A greater Shannon's index and reduced Simpson's index are indicative of greater diversity among microbial populations. At all time points (days 1, 7 and 14), the fecal microbiota of progeny derived from P1 dams was more homogenous when compared to P4 progeny (P < 0.001). With respect to microbial diversity, P1 progeny tended (P = 0.07; Shannon's) to have greater microbial diversity compared to P4 progeny on day 1, and on day 7, the reduction in microbial diversity in P1 progeny reached statistical significance (Shannon's: P < 0.05). There were no differences in microbial diversity among progeny derived from different dam parities (P1 v. P4) on day 14. These results suggest that microbial populations, and thus health status, may be affected by dam parity.

Introduction

It is possible that progeny health status is affected by factors including (but not limited to) animal stress, passive immunity, and susceptibility to pathogens. When passive immunity is low or fails, the piglet's health status decreases and may affect survivability. Therefore, receiving adequate colostrum in the first 24 hours after birth is extremely important.

Preliminary observations reported in the *2008 Nebraska Swine Report* suggest that passive immunity, and thus health status, may be affected by dam parity. In order to substantiate our preliminary observations, another experiment was initiated and the results were published in the *2009 Nebraska Swine Report*. Parameters evaluated in the 2009 experiment included litter performance and transfer of passive immunity and the results can be summarized as follows: 1) No differences in litter performance were observed among first parity (P1) compared to fourth parity (P4) dams with the exception of litter birth weight which tended ($P = 0.10$) to be greater for P4 compared to P1 dams; 2) Immunoglobulin (Ig) A concentrations during lactation in colostrum and milk samples tended ($P = 0.08$) to be greater in samples collected from P4 compared to P1 dams; and 3) P4 progeny had greater ($P < 0.05$) serum IgG concentrations compared to P1 progeny throughout lactation. These results confirmed our preliminary observations that passive immunity may be affected by dam parity.

More information is needed to understand how dam parity may affect progeny health status. Recently, considerable evidence accrued that the composition of the intestinal microbiota of an individual may be linked and used as indicators of gastrointestinal health status. Therefore, factors that may affect establishment of the pig's gastrointestinal microbiota are likely to affect animal performance and include host physiology, environmental exposure, and diet.

Denaturing gradient gel electrophoresis (DGGE) is a technique that is capable of discriminating among bacterial species and is a means by which patterns of change in microbial populations can be detected through space and time (Thompson et al., 2008). Increase in microbial diversity has been associated with increased ecosystem stability and resistance to pathogen invasion (Konstantinov et al., 2004). In addition, species diversity affects a number of processes in ecological communities, including productivity, stability, and susceptibility to invasive species (Hooper et al., 2005). Therefore, the objective of the current experiment was to evaluate fecal bacterial population changes among P1 and P4 progeny as a means to further our knowledge on the effect of dam parity on progeny health status.

Materials and Methods

Experimental Design

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use committee of the University of Nebraska–Lincoln. Dams (Large White × Landrace) utilized in the current study included P1

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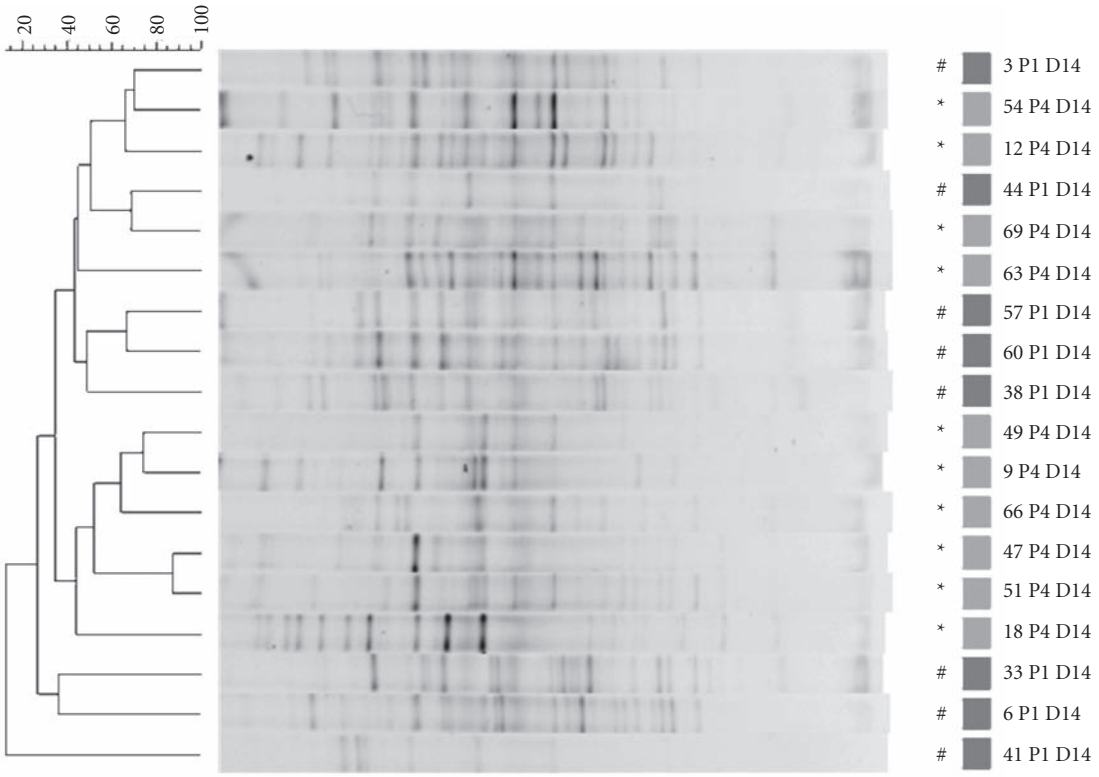

Figure 1. Dendogram derived from DGGE analysis of fecal bacterial community of piglets on day 14. UPGMA-type dendograms were constucted based on the similarity matrix resulting from Pearson's pair-wise comparisons of DGGE fingerprints. The red (#) squares represent P1 piglets and the green (*) squares represent P4 piglets.

gilts (n = 4) and P4 sows (n = 4). Dams were co-mingled and housed in stalls during gestation and moved to farrowing crates approximately five days prior to their expected farrowing date.

Fecal Sample Collection

Fecal samples were collected from six piglets from each litter (n = 4 from each dam parity, P1 and P4) on days 1, 7, and 14 following parturition. Fecal samples were stored in phosphate-buffered saline and frozen (-20°C) for further analyses.

Laboratory Analyses

Extraction of DNA from all fecal samples was carried out according to the methods described by Rasmussen et al. (2009). The resultant DNA was utilized for subsequent polymerase chain reaction (PCR) and DGGE analyses. Briefly, for investigation of the entire microbe population in fecal samples, PCR was performed by using universal primers to amplify the V3 region of the 16S rRNA gene. Denaturing gradient gel electrophoresis was performed as described by Walter et al. (2000). Denaturing gradient gel electrophoresis images were analyzed using BioNumerics software where the DGGE fingerprints were transformed to peak profiles and intensities of individual bands were determined as a percent peak surface area relative to the surface area of the entire molecular fingerprint of the sample. To determine the effect of dam parity, normalized fragment intensities of all bands in DGGE fingerprints were determined and compared among dam parities, which is partially depicted by the dendogram in Figure 1.

To determine the microbial diversity of the fecal DNA samples, Shannon's and Simpson's ecological indices were applied to the molecular fingerprints as described by Scanlan et al. (2006). Briefly, Shannon's diversity index was calculated using the formula shown below in which *pi* represents the proportions of a species *i* present in a sample (determined as the proportion of the band intensity with respect to the intensity of the entire fingerprint) of *n* different species (number of bands in the profile). Simpson's diversity index was calculated with the following formula in which *ni* represent the number of organisms belonging to species *i* (determined as proportion of the band intensity with respect to the intensity of the entire fingerprint) and *N*, the total number of organisms in the microbial population.

$$\text{Shannon's index} = \sum_{i=1}^{n} - pi * Ln\,(pi)$$

$$\text{Shannon's index} = \sum_{i=1}^{n} \frac{-\,ni^{*}(ni-1)}{N(N-1)}$$

Statistical Analysis

The GLM procedure (SAS Inst. Inc., Cary, N.C.) was used to analyze all parameters as a completely random design with repeated measures over time on each experimental unit. The model included terms for the fixed effects of parity and time and their interaction. Comparisons among dam parity and time were made only when a significant ($P < 0.05$ unless noted otherwise) F-test for the main effect or interaction was detected using the least significant dif-

Table 1. Similarity indices[a] of microbial populations in piglets (n=6).

	Parity 1[b]	Parity 4
Day 1	66.31 ± 3.12	34.74 ± 3.12
Day 7	55.69 ± 3.12	33.81 ± 5.93
Day 14	48.56 ± 3.12	33.42 ± 3.12

[a]Similarity indexes are calculated using the BioNumerics software, the DGGE fingerprints were transformed to peak profiles. Intensities of individual bands were determined as a percent peak surface area relative to the surface area of the entire molecular fingerprint of the sample.
[b]Parity x day $P < 0.05$.

Table 2. Diversity indices[a] of microbial populations in piglets (n=6).

	Parity 1		Parity 4		*P*-value	
	Shannons	Simpsons	Shannons	Simpsons	Shannons	Simpsons
Day 1	1.85 ± 0.21	0.30 ± 0.09	1.20 ± 0.21	0.37 ± 0.09	0.03	0.60
Day 7	2.80 ± 0.21	0.08 ± 0.09	2.30 ± 0.23	0.16 ± 0.10	0.12	0.40
Day14	2.65 ± 0.21	0.09 ± 0.09	2.43 ± 0.21	0.13 ± 0.09	0.46	0.73

[a]Diversity Indexes were calculated by comparing molecular fingerprints of DNA. A higher Shannon's diversity index represents more diversity. A lower Simpson's diversity index represents more diversity.

ference procedure. All means presented are least-squares means.

Results and Discussion

Denaturing gradient gel electrophoresis analysis revealed that there was substantial variation in gut microbiota composition among individual piglets (Figure 1). Similarity indexes for fecal microbial populations are depicted in Table 1. These coefficients represent the similarity of the microbial population within a group (P1 or P4). This analysis revealed that on days 1, 7, and 14, the microbial population of P1 piglets was more uniform when compared to P4 progeny ($P < 0.001$). Diversity indices (Shannon's and Simpson's) represent the differences of the bacterial species within the microbial population while each index weighs species richness and evenness slightly differently. Shannon's index incorporates species richness (number of species, or in this case, PCR-DGGE bands) and evenness (the relative distribution of species) and Simpson's index takes into account the number of species present, as well as the relative abundance of each species. An increasing Shannon's index signifies a more diverse microbial population, while a decreasing Simpson's index indicates a greater diversity. Differences in microbial populations with respect to microbial diversity using the Shannon's and Simpson's indices were determined and are represented in Table 2. Shannon's microbial diversity index was greater ($P < 0.03$) for P1 progeny on day 1 compared to P4 progeny, indicating that P1 piglets have greater microbial diversity compared to P4 progeny. There were no differences among parity found in Simpson's diversity index.

Collectively, differences in similarity indicate the presence of different bands (i.e., bacterial species) and differences in microbial diversity indicate an overall change in microbial community complexity. Therefore, with respect to P4 progeny, P1 progeny have greater similarity (i.e., fewer bacterial species) throughout the preweaning phase, but have greater diversity (i.e., number of bacterial species present and their relative abundance) through day 7 preweaning.

There are several factors which may account for the differences in gut microbial ecology that were observed in P1 and P4 progeny. Most importantly, the composition of sow milk may affect the bacterial population of its progeny. We have previously observed a numerically greater concentration of IgA in the colostrum and milk of P4 sows (*2009 Nebraska Swine Report*) and it is possible that this difference in immunoglobulins could account for some of the differences observed in microbial populations among progeny derived from different parities. Secretory IgA in colostrum is an important factor of microbial control in the piglet intestinal tract (e.g., control of pathogenic bacteria), as the piglets themselves have not yet developed a functional immune system. Therefore, IgA in milk is likely to affect the microbiota that become established in the gastrointestinal tract of the piglets. The greater IgA concentrations in P4 piglets would exert a greater selective force and could result in the lower microbial diversity in the gut of P4 progeny that we have observed in this study.

With respect to changes in the bacterial population (similarity and diversity), over time these changes could affect the functions that the microbial community supplies to the host and how the host responds to these changes. For example, changes in the microbial community could shift the production of short-chain fatty acids (i.e., butyrate) that may have an anti-inflammatory effect on the gut. Alternatively, changes in gut microbiota may affect the way in which the host responds to different microbes immunologically. That is, whether the host tolerates (immune response not initiated) or responds (immune response initiated with resultant inflammatory events) to changes in microbial populations.

Conclusion

Results from this experiment indicate that there are differences in microbial populations among progeny derived from different dam parities. However, more research is needed to determine how these changes may affect health status and growth performance.

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