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THE EFFECT OF DAM PARITY ON PROGENY GROWTH PERFORMANCE, PASSIVE IMMUNITY, AND GASTROINTESTINAL MICROBIOTA

Erin E. Hinkle
University of Nebraska-Lincoln, erin.hinkle2@gmail.com

THE EFFECT OF DAM PARITY ON PROGENY GROWTH PERFORMANCE, PASSIVE IMMUNITY, AND GASTROINTESTINAL MICROBIOTA

by

Erin E. Hinkle

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THE EFFECT OF DAM PARITY ON PROGENY GROWTH PERFORMANCE, PASSIVE IMMUNITY, AND GASTROINTESTINAL MICROBIOTA

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Advisor: Thomas E. Burkey

Previous research has shown that parity (P) 4 progeny have greater weaning weights and decreased microbial diversity compared to P1 progeny. Three experiments were conducted to evaluate litter performance, passive immunity, and fecal microbiota among P1 and P3 dams and their progeny. In experiment 1, 56 P1 and 49 P3 dams and their progeny's litter and growth performance, immunoglobulin (IgG and IgA) concentrations, and gut microbiota were evaluated. In experiment 2, 48 pigs per P were selected to determine growth performance, immunoglobulin (IgG and IgA) concentrations, and gut microbiota. In experiment 3, 8 dams per P were selected. At birth, piglets were fostered to P3 or P1 dams, creating 4 treatments 1) Parity 1 dam with P1 progeny 2) Parity 1 dam with P3 progeny 3) Parity 3 dam with P1 progeny 4) Parity 3 dam with P3 progeny to determine if passive immunity or *in utero* growth had more of an effect on growth performance. Few differences were observed between parities in litter performance. Progeny BW was increased for P3 progeny compared to P1 dams throughout lactation and the nursery period (d 0 to 63 of age), irrespective of crossfostering. P3 progeny had increased ADG and ADFI during all phases of the nursery period and overall compared to P1 pigs. Progeny derived from P3 dams had greater serum IgG concentrations compared to P1 progeny during lactation. Circulating IgA concentrations were greater in

P3 progeny on d 0 of lactation, but were increased in P1 pigs compared to P3 pigs during the nursery period. Despite dam parity's effect on the immune system, dam parity did not affect gut microbiota. During the lactation period, pigs had similar ADG despite compromised immunity of P1 raised progeny. Therefore, in utero growth and development had a larger impact on growth rate than passive immunity. Growth performance, body weights, and immune parameters of pigs are influenced by dam parity.

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Chapter 1

Genetics, Environment, and Diet effects on the Composition of Gut Microbiota and Metabolic Disease in Human and Animal Models

Erin E. Hinkle, Ph.D.

SUMMARY

The gut microbiota effect several systems in the body including nutrient digestion, absorption, and metabolism as well as immune system development, sustainability, and protection from pathogens. Due to the large influence of gut microbiota, potential influences on the gut microbiota have been of great interest. Genetics, the environment and diet all effect the composition of the gut microbiota. In particular, the diet has the largest effect on gut microbial composition. The early diet can have a large impact on the immune system, which may affect disease prevalence later in life. In adulthood, high fat, carbohydrate (including fiber), and protein diets each affect the gut microbiota differently. Understanding how genetics, the environment, and differences in diet change the gut microbiota and its effects on the host are essential to increase our knowledge on health and disease.

INTRODUCTION

The gastrointestinal tract (GIT) is involved in several body processes including, but not limited to, nutrient digestion, absorption, and metabolism and the host immune system development, sustainability, and protection from pathogens (Clemente et al., 2012). Collaborating in the maturation and maintenance of the GIT are the millions of microbes that reside in it. The development, or underdevelopment, of this organ and its microbiota can lead to numerous chronic diseases and disorders. Therefore, knowledge of the interactions of the GIT and its microbiota with nutrient utilization is essential for advancement in feeding our livestock species to increase growth performance and health status as well as making enhancements in human disease.

Millions of microbes live in the GIT. The most dominant phyla for all mammalian species are Firmicutes, Bacteroidetes, and Actinobacteria. Abundance of each of the phyla varies by species and environment (Lesar and Molbak, 2009). Variation also is observed between species at the genus level. Differences between individuals can be quite broad, though more related than variation among species. Dissimilarity can play a major role on nutrient utilization, as well as gut and immune system development. Despite the variation we are able to determine how genetics, environment, and diet may influence gut microbiota (GM) development. Exploring the types of bacteria in the gut and how they respond to certain stimuli can lead us to a better understanding of how the gut reacts to changes in the microbiota and how this might mediate or cause diseases such as obesity, type 2 diabetes, colorectal cancer, autoimmune diseases, allergies, and coronary heart disease in humans, as well as increased health status and growth performance in pigs.

This review will explore the impacts of genetics, environment, and diet on the GM of human and animal models. Special attention will be paid to effects on the gut microbiota of pigs; however, limited research is available. It is therefore important to understand influences of the gut microbiota in all species to understand how the gut microbiota may influence nutrient utilization, gut and immune system development.

GENETICS

The role of genetics in determining the composition of the gut microbiota has been highly controversial. While several studies reveal genetic effects, other factors (such as environment and diet) play a more prominent role in determining the composition of the gut microbiota. Genetic effects, however, should be established to differentiate between environmental and genetic effects on gut microbiota and their role in nutrient metabolism and disease.

Studies that compare the gut microbiota of twins, litter mates, and inbred mice have given us an idea of how genetics can influence the gut microbiota. The effect of genetics varies greatly by species and even by mouse genetic strains. For example, when 10 different genetic strains of mice (housed in the same environment and fed the same diet) were compared, analysis of operational taxonomic units (OTU) showed clustering by genetic strain (Campbell et al., 2012). Similar results were observed by Friswell and colleagues (2010). Differences between strains are largely affected by the amount of Firmicutes and Bacteroidetes (Campbell et al., 2012). In humans, several studies have compared the gut microbiota of families including monozygotic (MZ) and dizygotic (DZ) twins. The gut microbiota of twins (MZ and DZ) (Zoetendal et al., 2001) and families (Turnbaugh et al., 2009a) are more similar than genetically unrelated individuals; however there was no difference in similarity between MZ and DZ twins. This suggests that environment or diet may have a greater impact on the composition of GM.

Genetic effects on GM have not been detected in all species. A study comparing the gut microbiota of families of chimpanzees reported no genetic influence on gut microbiota (Degnan et al., 2012). Also, comparing littermates of pigs which where cohabitated with other litters showed that cohabitation had a much larger effect on GM than genetics (Thompson et al., 2008).

Despite these conflicting results, recent evidence has reported heritability of the GM in mice (Benson et al., 2010). Analysis of quantitative trait loci (QTL) has revealed correlations to a core microbial community. Selection for GM composition at the genetic level is focused at the tips of the phylogenetic tree, or at the genus and family levels. Some of the taxa linked to QTL are Bacteriodetes, Clostridia, Bacilli, as well as Coriobacteriaceae, Turicibacter, and *Helicobacter* (Benson et al., 2010). No QTL were identified with *Lactobacillus*, however, when analyzed at the species level, *L. johnsonii/gasseri* group identified with 2 QTL (Benson et al., 2010). An additional study (McKnite et al., 2012) took the analysis one step further and identified candidate genes that could be influencing the GM. Examples of this include, a QTL identified for Prevotellaceae could be a gene that encodes an anti-inflammatory cytokine (*Tgfb3*) with a potential role in modulating intestinal barrier function and commensal bacteria tolerance and a QTL region identified for increasing the proportion of Bacteroidetes is also known to encode interferon alpha, beta, zeta, and epsilon (McKnite et al., 2012).

Interestingly, the bacteria that are most dominantly linked to QTL are also linked to disease. For example, Coriobacteriaceae, *Turicibacter*, and *Barnesiella* are all linked to diseases in the murine model (which was used in the study; Benson et al., 2010). The QTL identified with these organisms overlap with known QTL for diseases (*Turicibacter* QTL overlaps QTL for murine hepatocollular carcinomas, QTL for Coriobacteriaceae overlaps QTL for Scc9 locus which is associated with murine susceptibility to colon tumors; Benson et al., 2010). Further studies of genetic impacts on the GM could increase our understanding of the GM effect on health and disease.

ENVIRONMENT

The environment has more effect on the composition of the gut bacterial community than genetics. In fact, environmental factors have played such a large part on the development of the GM that it masked any genetic influences for a time. Mice of different genetic strains that are housed in the same cages will have more similar GM than mice of the same genetics if grouped before bacterial maturity is reached (Friswell et al., 2010; Campbell et al., 2012). Also, mice of the same strain that are reared at different institutions will cluster by location over genetic strain (Friswell et al., 2010). In pigs, littermates raised under similar environments and similar diets, but housed in different pens have a GM that clusters by pen and not by littermate, which may make environment more influential on GM than genetics in pigs; however, more research is necessary for this to be determined (Thompson et al., 2008).

Environmental influences have also been observed when comparing humans from different geography. Variation in GM has been reported between adults and elderly in European countries (Mueller et al, 2006). *Eubacterium rectal-Clostridium coccoides, Bacteroides-Prevotella, Faecalibacterium prausnitzii,* and *Atobium* concentrations differed by European country and age and *Bifidobacterium* was affected by European country (Mueller et al., 2006).

Children from Africa had increased Bacteroidetes and decreased Firmicutes compared to European children. In particular, *Prevotella* and *Xylaibacter* were enriched in African children (De Filippo et al., 2010). Comparing children and adults from the U.S. to Amerindian or Malawian children and adults resulted in clustering of GM by age and geography(Yatsunenko et al., 2012). Infants that were breast-fed clustered together regardless of country, while adults clustered by country (Yatsenenko et al., 2012).

Although this variation in GM is geography related it is likely differences seen here are also related to differences in diet, as less developed countries consume more fiber than more developed countries, also termed a Westernized diet, which is higher in protein and fat.

Other studies have compared the GM of pigs raised in different environments. Pigs raised on a commercial farm had decreased Lactobacilli in all gut segments and decreased yeast in the cecum and colon compared to pigs raised in an experimental research environment (Janczyk et al., 2010). Good versus poor sanitary conditions revealed more *Lactobacillus* and *Enterobacteria* and less anaerobic sulfite bacteria in poor sanitary conditions (Montagne et al., 2012). Pigs raised indoor have also been compared to pigs raised outdoors. Pigs raised outdoors had more Firmicutes (mostly *Lactobacillus*) compared to pigs raised in an indoor (commercial farm) environment (Mulder et al., 2009). To take it a step further, pigs raised in each of these environments were transferred to isolators which increased Bacteroidetes and Proteobacteria while decreasing Firmicute numbers regardless of the pigs original environment (indoor or outdoor; Schmidt et al., 2011). Comparing pigs raised in these different environments may lead us to a better idea of how sanitary conditions may affect the GM and through the GM, the immune system, which may lead to allergies and allergic diseases; essentially testing the hygiene hypothesis.

DIET

The most influential factor on the GM is diet. Differences in diet have been found to play a very large role in the composition of GM. The effects of diet of the GM start very early with the introduction of breast-milk or formula (Table 1), as breast-fed babies have increased amounts of Bifidobacteria than formula fed babies. These early effects on development may last a lifetime, as GM affect immune systems development. In adulthood, the amount of fat (Table 2), carbohydrates (fiber; Table 3) and protein (Table 4) consumed can influence the composition of bacterial phyla. Consuming a high fat diet increases Firmicutes while simultaneously decreasing Bacteroidetes, which has been associated with several diseases such as obesity and type 2 diabetes. Adding fiber to a diet can greatly influence GM composition based on the type of fiber. The addition of prebiotics has been of great interest recently and may be used to manage certain diseases, including postweaning diarrhea in pigs.

Early Development/Breast-fed vs. Formula-Fed

The GIT is considered sterile when a neonate is born. Bacteria start to colonize the GIT almost immediately after birth. The type of bacteria that start to colonize is dependent on the mode of delivery. Immediately after birth, neonates delivered vaginally develop a bacterial community that is similar to the mother's vaginal bacterial community while neonates delivered via cesarean section develop a bacterial community similar to the bacteria that colonize the mother's skin (Dominguez-Bello et al., 2010). Vaginally delivered neonates GM were dominated by the taxa *Lactobacillus, Prevotella, Atopobioum*, or *Sneathia* spp. Caesarean delivered neonates GM were dominated by *Staphylococcus* spp. (Dominguez-Bello et al., 2010). Differences in delivery mode in bacterial colonization can also be observed at 1 month of age. Compared to vaginal delivery, cesarean delivered neonates had decreased colonization rates of bifidobacteria and greater amounts of *Clostridium difficile* and *E coli* (Penders et al., 2006). At 6 weeks of age, *Bacteroides* and *Atopobium* were increased and *C. coccoides* group and *Streptococcus* group decreased in vaginally delivered infants compared to c-section infants (Fallini et al., 2010). Differences in GM between modes of delivery may even continue up to 7 years of age (Salminen et al., 2004).

Another factor that plays a major role in the development of the GM of infants is diet; in particular breast-fed versus formula fed (Table 1). The most abundant bacteria in the infant gut are bifidobacteria (Yoshiok et al., 1983; Harmsen et al., 2000; Hopkins et al., 2005;). This was also observed in the piglet gut (Li et al., 2012) which is commonly used as a model for the infant gut. The concentration of bifidobacteria colonizing the gut is increased for infants that are breast-fed compared to those that are formula-fed (Yoshioka et al., 1983; Harmsen et al., 2000; Hopkins et al., 2005; Fallini et al., 2010) Formula-fed infants have greater concentrations of *Bacteroides* (Harmsen et al., 2000; Hopkins et al., 2005; Penders et al., 2006; Fallini et al., 2010), *Clostridium* spp., especially *C. difficile* (Harmsen et al., 2000; Hopkins et al., 2005; Penders et al., 2005, 2006; Fallini et al., 2010), and enterobacteria (Yoshioka et al., 1983; Harmsen et al., 2000; Hopkins et al., 2005; Penders et al., 2006). Similar differences have been found in piglets that are sow-reared versus formula-fed, although the dominant genus has not been consistent. Porokyo et al. (2010, 2011) found that sow-reared piglets were dominant in *Prevotella, Oscillibacter,* and *Clostridium*, while Li et al. (2012) reported increased concentrations of bifidobacteria in sow-reared pigs compared to formula-fed piglets. Formula-fed piglets in the studies by Porokyo et al. (2010, 2011) had increased concentrations of *Bacteroides, Parabacteroides,* and *Alistipes* compared to sow-reared piglets. Li et al. (2012), however, reported increased concentrations of *Clostridium* cluster IV and XIVa and *Bacteoroides vulgatus* compared to sow-reared piglets. The differences in these two studies could be due to the differences in experimental methods. Li et al. (2012) used DGGE and real-time qPCR techniques that are specific for bacterial genera, while Porokyo et al. (2010, 2011) pyrosequenced total bacterial DNA and was therefore able to look at a more detailed bacterial population.

These differences seen in GM of breast-fed and formula-fed infants are likely due to the increased concentrations of human milk oligosaccharides (HMO) in breast-milk. Several bifidobacteria have been shown to utilize HMO (Liepke et al., 2002; Lo Cascio et al., 2010; Shen et al., 2011; Garrido et al., 2012), with *B. infantis* preferentially utilizing HMO and *B. longum* preferring plant based oligosacchardies (Garrido et al., 2012). Both of these *bifidobacteria* have been prominently identified in the infant gut. Recently, *Bacteroides* and the clostridium cluster XIVa have been shown to utilize HMO as well (Marcobal et al., 2010; Shen et al., 2011; Li et al., 2012). The addition of oligosaccharides, in particular fructooligosacchrides (FOS) and galactooligosaccharide (GOS), to formula increases the concentration of bifidobacteria in the feces of infants (Penders et al, 2006; Li et al., 2012).

Recently, the gene expression of the microbes of sow-reared piglets and formula-fed piglets has been studied (Porokya et al., 2010). Several of the major systems were not different between the two diets, including a majority of carbohydrate metabolism systems. Differences in diet were observed in only two carbohydrate systems including increased transcript utilization of L-arabinose and the sugar alcohol mannitol in fomula-fed pigs. Sow-reared piglets had increased expression of galactose mutarotase (metabolizes lactose) and transketolase (phage resistance, LPS metabolism, and flagellar motor function), the latter of which is possibly linked to Bifidobacteria. This study however, did not detect any Bifidobacteria in cecal samples. This could be due to the lack of the use of a primer that detects bifidobacteria. Arginine metabolism, proline biosynthesis, glycine cleavage, and histadine degradation was increased in sow-reared piglets while formula-fed piglets were enriched with transcripts for glycine and serine utilization and aromatic amino acid degradation. Despite these observations, differences in gene expression could not be linked to specific bacteria; therefore, more research is necessary.

Breast-feeding for at least 4 months has been linked to several health benefits. Short term benefits included decreased incidence of diarrhea (Le Huerou-Luron et al., 2010) and gastrointestinal disease (Rebhan et al., 2009). Part of this is likely due to the structure of HMO which are similar to cell surface glycans, which inhibits binding of pathogens to cell surfaces, therefore providing pathogen protection (Newburg, 2009). There has also been a link to decreased incidence of type 2 diabetes (Owen et al., 2006) and obesity (Harder et al., 2005) with breast-feeding, though the underlying mechanisms for these diseases remain unclear. In a meta-analysis, breast-fed subjects, later in life, had decreased fasting insulin concentrations and lower preprandial blood glucose and insulin concentrations than formula fed subjects (Owen et al., 2006). The link to obesity could be due to a satiation effect. Infants, at one year of age, that are breast-fed were more likely to develop a satiety response than formula-fed infants (Dewey and Lonnerdal, 1986; Brown and Lee, 2012). At the same age infants are also leaner (Dewey et al., 1993; Hediger et al., 2000) which could lead to a self-regulation mechanism in later life. An increase in bifidobacteria colonization in the gut could be part of this underlying mechanism as it has been linked to a higher incidence of remaining at a normal weight in 7 year old children (Kalliomaki et al., 2008). More research is needed to determine if there is an actual link and possible mechanisms of early bacteria colonization to diabetes and obesity.

Another benefit of breast-feeding is linked to a decreased incidence of allergies and asthma. Bifidobacteria has been shown to induce immune-tolerance which decreases the incidence of allergies and asthma. This topic is not the focus of this review, but has recently been reviewed (Frei et al., 2012).

Fat

As mentioned above, eating a high-fat diet can alter the GM (Table 2). These changes can occur hours after consuming a meal and may become constant after chronic consumption of a high-fat diet. Mice bacterial communities shifted 1 day after the consumption of a high fat meal (Turnbaugh et al., 2009b). After only 7 days on a high-fat diet, these changes became stable (Turnbaugh et al., 2009b). These changes included an increase in bacteria in the class Erysiphelotrichi and Bacilli which are member of the Firmicutes phylum and a decrease in the phylum Bacteroidetes (Turnbaugh et al., 2009b). Several studies have looked at the effect of a high-fat diet on GM. In general, mice studies have revealed, at the phylum level, an increase in Firmicutes and a decrease in Bacteroidetes (Turnbaugh et al., 2008, 2009b). Within the Firmicutes increases have been detected in Clostidiales (Hildebrandt et al., 2009; De La Serre et al., 2010), and mollicutes (Turnbaugh et al., 2008). Within the Bacteroidetes phylum, Bacteroides (Cani et al., 2007; Cani et al., 2008; Hildebrandt et al., 2009) and Prevotella (Cani et al., 2008; Hildebrandt et al., 2009) have decreased. Bifidobacteria has also been shown to decrease when fed a high-fat diet (Cani et al., 2007, 2008; Brinkworth et al., 2009).

These differences are not harmonious across studies, as some experiments have shown increases in Bacteroidetes and decreases in Firmicutes (De La Serre et al., 2010; Wu et al., 2011; Devkota et al., 2012) or an overall decrease in total bacteria (Dewulf et al., 2011). This could be due to bacteria detection methods, subject studied, as well as percentage and type of fat used in the diet. A majority of high-fat diet studies have used lard as the principle component of fat (Cani et al., 2007, 2008; Dewulf et al., 2011) or did not report fat type (Turnbaugh et al., 2008; 2009; Hildebrandt et al., 2009; De La Serre et al., 2010), however, a recent study looked at the effects of saturated milk fat or polyunsaturated fatty acids (PUFA) to a low fat diet (Devkota et al., 2012). Milk fat and PUFA increased Bacteroidetes and decreased Firmicutes, which is opposite of the effects of lard on GM. Interestingly, milk fat also increased the pathogen *Bilophila wadsworthia*, which flourishes in the presence of the taurine-conjugated bile acid (Devkota et al., 2012). This suggests that the type of fat in the diet changes the environment of the gut which then alters the GM.

The effects of high-fat diets on GM have been studied to determine the role of GM on obesity and diabetes. While a possible link has been established between GM and obesity, particularly in genetically obese individuals (reviewed by Sanz et al., 2010), a high-fat diet does not always cause obesity, but does change the GM (de La Serre et al., 2010). These changes in the GM can affect functions of the intestine. A high-fat diet increases the percentage of Gram-negative bacteria in the gut while decreasing Gram-positive bacteria (Cani et al., 2007). This increases the concentration of lipopolysaccharide (LPS) in the intestine, which increases the presence of endotoxins (Cani et al., 2007, 2008; Erridge et al., 2007), increases intestinal permeability (Cani et al, 2008; de La Serre et al., 2010; Muccioli et al., 2010), and increases inflammation by activating toll-like receptor 4 (TLR4; de La Serre et al., 2010). Activating TLR4 creates a cascade of events inside the body that can cause chronic inflammation which can lead to obesity, type 2 diabetes, cardiovascular disease, and metabolic syndrome (Nakamura and Omaye, 2012).

In order to reverse the effects of diet on these diseases several methodologies have been studied, including adding fiber or probiotics to the diet. Supplementing a high-fat diet with inulin or β-glucans ameliorated some of the effects of a high-fat diet, including decreased weight gain and fat pad weight (Dewulf et al., 2011; Arora et al., 2012). This could be due to the increase in bifidobacterium observed in the GM. In fact similar results have been observed with the addition of *Bifidobacterium* ssp. (An et al., 2011; Chen et al., 2012).

Carbohydrates

Fiber/Prebiotics

There are several types of fiber and each has a different effect on the GM (Table 3). Prebiotics are defined as the “selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host” (Roberfroid et al., 2010). The most researched fibers include inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and resistant starch (RS) and each is considered a prebiotic.

The effect of fiber on the GM is dependent on the type of fiber. Inulin and FOS are short chain fructans that have varying degrees of polymerization (DP; FOS 2-20 DP, inulin 3-60 DP). Inulin and FOS are shown to have bifidogenic effects *in vitro* (Van de Wiele et al., 2007) humans (Gibson et al., 1995; Bouhnik et al., 1999; 2007; Rao, 2001; Touhy et al., 2001; Kruse et al., 1999; Brighenti et al., 1999) and rodents (Van der Abbeele et al., 2011). The addition of inulin and FOS to pigs did not change GM (Mikkelsen et al., 2003; Mikkelsen and Jensen, 2004; Janczyk et al., 2010) or increased the amount of lactic acid bacteria (LAB; Lui et al., 2012).

The prebiotic, GOS, is generally added to baby formula. As a byproduct of lactose hydrolysis, GOS is composed of a mixture of glucose and galactose, with at least 2 galactose units and a glucose terminal end that is between 2 and 8 saccharide units (Torres et al., 2010). It has been studied in adults and weaned pigs. In adults and *in vitro* GOS increased the concentration of bifidobacteria compared to a placebo (Walton et al., 2012) and increases the bifidogenic effect with an increase in the dosage (Davis et al., 2010, 2011). No differences in GM we detected when weaned pigs were fed GOS (Mikkelsen et al., 2003; Tsukahara et al., 2010).

Resistant starch is defined as starch that restists degreadation in the small intestine and is available for fermentation in the colon. There are 4 types of RS: type 1, undisrupted plant structure as whole or partially milled grain; type 2, starch in granules of partially crystalline form such as ungelatinized granules; type 3, retrograded starch, such as cooked potato, bread, and cornflakes; and type 4, chemically modified food starches and dry-heated starch (Kumar et al., 2012). *In vitro* models show a shift in the microbiota of the colon with increases in *Bifidobacterium* and *Atopobium* spp. (Lesmes et al., 2008). In rats, all types of RS increases the growth of bifidobacteria (Kleesen et al., 1997; Silvi et al., 1999; Wang et al., 2002; Conlon and Bird, 2009; Young et al., 2012). Type 2 RS decreased Bacteroides and increases total culturable bacteria, lactobacilli, streptococci, and enterobacteria in the cecum of rats (Kleessen et al., 1997). Rats colonized with a human gut microflora saw a increase in lactobacilli and bifidobacteria in the cecum (Silvi et al., 1999). In the aged mouse, RS type 2 increased Bacteroidetes and the increase was due to unclassified members of the phylum (Tachon et al., 2012). Similarly, conventionally raised rats saw increases in Bacteroidetes and Actinobacteria with proportional decreases in Firmicutes in the colon (Young et al., 2012). The increases in the phylum were due to the growth of Porphyromanadaceae and Bifidobacterium, respectfully, and a decrease in clostridium (Young et al., 2012). Similar results have been observed in humans, as RS type 4 decreased Firmicutes and increased Bacteroidetes and Actinobacteria (Martinez et al., 2010). One difference from the mouse/rat models shows an increase in Ruminoccocus (Martinez et al., 2010; Abell et al., 2008). Mixed results have been observed in pigs. Castillo et al. (2007) reported no differences in GM of pigs fed RS, while Metzler et al. (2009) reported an increase in Lactobacillus.

The addition of fiber to a diet has been shown to have several health benefits, including decreased incidence of colorectal cancer, coronary heart disease, obesity, and type 2 diabetes. How fiber decreases these incidences is still unknown, but several possibilities exist. These include increased production of short chain fatty acids (SCFA), increased rate of transit through the intestine, increased binding of bile salts to carcinogens, and increased antioxidants (Lattimer and Haub, 2010). Of these possible mechanisms the production of SCFA is the most associated to changes in the GM. The SCFA produced at the greatest concentrations in the gut are acetate, propionate, and butyrate. These three SCFA are usually produced at a ratio of 60:25:15, respectfully, however, changes in this ratio may be the key to mediating the risk of disease.

Acetate, the most produced SCFA in the colon, is used by several parts of the body, including the liver and muscle. A positive effect of acetate is its ability to increase immune defenses by increasing immune cell recruitment. Negative effects of acetate is its association with increased lipid metabolism (Vipperla and O'Keefe, 2012) and possible role in the incidence of obesity (Turnbaugh et al., 2006). Acetate is also involved in the synthesis of cholesterol (Vipperla and O'Keefe, 2012), which over-synthesis can lead to coronary heart disease. Acetate is produced by most commensal bacteria in the gut, including Bifidobacteria and Lactobacillus. This indicates that these bacteria have some other mechanism as a probiotic. It is possible that cross-feeding occurs, as it has been observed that *Bifidobacterium adolescentis* growing in a medium containing FOS products will feed *Eubacterium hallili*, *Anerostipies caccae*, and *Roseburia* which then produce butyrate (Belenguer et al., 2006). Increased butyrate production has several health benefits as described below.

Propionate has the opposite effect of acetate on lipid metabolism, as it inhibits cholesterol synthesis, inhibits lipolysis, inducing lipogenesis in adipose tissue and reduces fatty acid synthesis in the liver (Vipperla and O'Keefe, 2012). These effects decrease plasma fatty acid levels which reduce inflammation and improve insulin sensitivity. Propionate also inhibits the NF-κB pathway, which also inhibits inflammation (Macfarlane and Macfarlane, 2011). Another anti-obesity effect of propionate is its ability to increase satiety (Macfarlane and Macfarlane, 2011). These effects of propionate on the body make it an attractive SCFA to increase production of in the colon through prebiotics. Consumption of inulin has also been shown to increase propionate production (Juskiewicz et al., 2007; van de Wiele et al., 2007; Grootaert et al., 2009; Van den Abbeele et al., 2011).

Butyrate is the chief energy source for colonocytes, making it possibly the most important SCFA. Butyrate has been shown to be the most beneficial in the prevention of colorectal cancer as it induces differentiation and apoptosis of colonocytes (Fung et al., 2012). Butyrate has also been shown to increase satiety and has several anti-inflammatory effects including mediating tumor necrosis factor – α (TNF-α) and nitric oxide (NO), and suppressing the NF-κB pathway (Vipperla and O'Keefe, 2012). Bacteria classified in the Clostridia cluster XIVa and IV of Firmicutes are considered the chief butyrate producers (Vipperla and O'Keefe, 2012). Several studies have shown that RS induces the production of butyrate (Wang et al., 2002; Lesmes et al., 2008; Conlon and Bird, 2009; Fung et al., 2012). FOS also increases butyrate production (Shim et al., 2005). Long term inulin consumption in humans did not change SCFA ratios (Bouhnik et al., 2007); however, consuming inulin did increase butyrate production in rats (Juskiewicz et al., 2007; van de Wiele et al., 2007). In humans over 50 years of age, GOS increased butyrate production (Walton et al., 2012); however, GOS had no effect on SCFA production in pigs (Tsukahara et al., 2010).

In the pig, fiber may reduce the incidence of post-weaning diarrhea. The main mechanism behind this is an increase in bacterial fermentation, which increases SCFA production and decreases gut pH. The lower gut pH inhibits the growth of pathogens, such as *Esherichia coli*, *Salmonella*, and *Clostridium difficile* (Niba et al., 2009). The addition of oligosaccharides might also decrease the incidence of diarrhea through the mechanism mentioned previously in breast-fed vs. formula fed infants. The oligosaccharides have a similar structure to cell surface glycans which inhibits binding of pathogens to cell surfaces (Newburg, 2009). Despite the possible mechanism, contradictory evidence exists and more research is needed.

Other Fibers

Fermentable carbohydrates have also been explored as a possible additive to nursery diets to control the incidence of diarrhea in weaned pigs. The type and amount of fermentable carbohydrates varies with each experiment (Table 3), however, sugar beet pulp (SBP) is common in most of the studies. Other fermentable carbohydrates that have been added include wheat bran, wheat middlings, soybean hulls, sunflower seeds, and potato starch. Each of these ingredients varies in insoluble and soluble fiber, but in a majority of the studies insoluble fiber was the dominant fiber source. High fiber diets reduced the growth performance of weanling pigs (Bikker et al., 2006; Montagne et al., 2012) or had no effect (Jeaurond et al., 2008; Pieper et al., 2012). High fiber diets effect on the GM differed with each fiber source. The addition of wheat middlings, sunflower seeds, potato starch, and SBP increased lactobacilli (Bikker et al., 2006; 2007) without any effects on *E. coli* prevalence (Bikker et al., 2006). In contrast, Lactobacilli concentrations did not change with the addition of wheat bran and SBP to the diet; however, bacteroides, *Clostridium leptum* and *C. coccoides* increased (Pieper et al., 2012). Soybean hulls and SBP decreased aerobic mesophilic bacteria, anerobic sulfite-reducing bacteria, and *Enterococcus* (Montagne et al., 2012). Feeding only SBP decreased *Clostridia* (Jeaurond et al., 2008) or had no effect (Castillo et al., 2007). Studies that mainly added soluble fiber to the diet have also been conducted. Konstantinov et al., (2003) reported an increase in *Ruminococcus* when SBP and FOS were added to the diet. When inulin, lactulose, wheat starch and SBP were added *Lactobacillus reuteri* and *L. amylovorus* concentrations increased (Konstantinov et al., 2004).

Another fiber that is added to diets at weaning, fenugreek seed, has been less well studied. The addition of Fenugreek seed to a weanling pig diet did not affect pig performance, but did increase *Lactobacillus* and clostridium cluster I concentrations while lowering *Escherichia, Hafnia,* and *Shigella* concentrations (Zentek et al., 2012). Fenugreek seed contains 32% insoluble and 13.3% soluble fiber with high concentrations of galactose and mannose (Zentek et al., 2012), which could be used to bind *E. coli* and Salmonella. Despite these changes in GM, adding fiber to the diet of weanling pigs may not be beneficial due to its possible adverse affects on growth performance.

Simple Sugars

Most bacteria use simple sugars, such as glucose, galactose, and lactose as sources of energy. Recently, there has been evidence that lactose may be a potential prebiotic as it could stimulate beneficial bacteria growth in the colon (Table 3). In the pig, feeding a 40% dried whey diet, approximately 30% of lactose will reach the colon (Kim et al., 1978). Pigs inoculated with lactose as well as *Lactobacillus acidophilus* resulted in an increased growth rate of all lactobacillus ssp., not just *L. acidophilus* (Pollman et al., 1980). When high (215 g lactose/kg) versus low levels of lactose (125 g lactose/kg) were compared, high levels of lactose increased *Bifidobacteria* populations (Pierce et al., 2007). When *Lactobacilli* sp. concentrations were compared in weaned pigs, *L. johnsonii*, and *L. reuteri* growth was induced with the addition of lactose, while *L. delbrueckii* decreased (Tran et al., 2012). More research is needed to determine the prebiotic role of lactose in pigs.

The effects of lactose as a potential probiotic in humans are likely to exert a greater effect on lactose maldigesters, in small doses, as more lactose will reach the colon for fermentation than lactose digesters. This prebiotic effect, however, is still in the immature stages as the results have been inconsistent. In a survey study, comparing digesters and maldigesters lactose intake with fecal microbial populations, no differences were found (Szilagyi et al., 2009). When digesters and maldigesters were provided 25 g of lactose twice a day, maldigesters bifidobacteria counts increased, with no effect observed on lactobacilli counts (Szilagyi et al., 2010). In infants with allergies to cow's milk, the addition of lactose to their diet increased bifidobactia and lactic acid bacteria concentrations while also decreasing bacteroides/clostridia (Francavilla et al., 2012). These changes in GM due to lactose may have a positive role in host health and metabolism, especially in maldigesters.

Protein

Surprisingly little research has been done on the effects of a high protein diet on the GM in monogastric animals (Table 4). Some research has looked at increasing crude protein (CP) levels in pigs, but a majority of the research that has been conducted looked at the carnivorous diet of the feline. In weaned pigs, increasing levels of CP increased *E. coli* and decreased lactobacilli populations when lactose levels were low but had no effect when lactose level were high (Pierce et al., 2007). *Bifidobacteria* also decreased with increasing levels of CP (Pierce et al., 2007). A high protein diet in adult cats increased *C. perfringens* and decreased bifidobacteria (Lubbs et al., 2009). In kittens, a high protein diet decreased *Bifidobacteria, Lactobacillus* and *E. coli* amounts in fecal samples (Vester et al., 2009). A more detailed look at high protein effects on kitten GM revealed increased *Clostridium, Faecalibacterium, Ruminococcus, Blautia,* and *Eubacterium* and decreased *Dialister, Acidominococcus, Bifidobacteria, Magasphaera,* and *Mitsuokella* compared to moderate protein diets (Hooda et al., 2012). It is evident from these studies that a high protein diet influences the GM, but more research is needed to determine proteins effects on host metabolism and health.

Glycoproteins have also been shown to affect the GM, in particular with their capacity to decrease the incidence of *E. coli* adhesion to the gut (Rhoades et al., 2005; Hermes et al., 2011; 2012). Glycoproteins are composed of a protein bound to a glycoconjugate, such as sialic compounds. Sialic compounds are also highly abundant in human milk oligosaccharides, which are likely responsible for the glycoproteins anti-adhesion properties. In addition to decreaseing *E. coli* concentrations, glycoproteins have also been shown to increase *Bifidobacteria* and *Lactobacillus* amounts *in vitro* (Idota et al., 1994; Bouhallab et al., 1994) and in the pig (Hermes et al., 2012).

CONCLUSION

Genetics, environment, and diet each affect the GM of human and animals in unique ways. Genetics role on the composition of the GM currently seems to be minor, but as more evidence is collected, understanding the impact of host genetics on GM could lead to increased understanding of the GM impact on diseases such as ulcerative colitis and Crohn's disease. The greater impact of environment on GM has lead to a better understanding the role that sanitary conditions role has on GM development and the hygiene hypothesis. The greatest influences on the GM are diet. Nutrients provided early on play a large role on development and may have lasting effects. Consuming a high fat diet induces inflammation and disease through changes in the GM, but this may be ameliorated by the addition of fiber or prebiotics to the diet.

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Table 1. Early Colonization of infant gastrointestinal tracts influenced by a breast-fed or formula-fed diet.

Source	**Detection Method**	**Site**	**Findings**	**Reference**
Infant	FISH	Feces	Bifidobacteria are the dominant bacteria in breast-fed infants. Formula-fed infants had higher concentrations of *Bacteroides, C. Coccoides,* and *Lactobacillus.*	Fallini et al., 2010
Infant	Cultured FISH	Feces	Bifidobacteria are the dominant bacteria in breast-fed infants, with lactobacilli and streptococci also present. Formula-fed infants have similar amounts of *Bacteroides* and bifidobacteria, with staphylococci, *E. coli*, and clostridia also present.	Harmsen et al., 2000
Infant	RT-PCR	Feces	The concentration of bifidobacteria colonizing in the gut is increased for infants that are breast-fed. Formula-fed infants have higher concentrations of *Bacteroides.* Formula-fed infants have higher concentrations of bacteroides, *C. coccoides*, and enterobacteria.	Hopkins et al., 2005
Infant	RT-PCR	Feces	Bifidobacteria similar between breast- and formula-fed infants. Formula-fed infants have higher concentrations *Clostridium* spp., especially *C. difficil*,. and enterobacteria.	Penders et al., 2005, 2006
Infant	Cultured	Feces	Bifidobacteria are the dominant bacteria in breast-fed infants, while enterobacteria are dominant in formula-fed infants a 6 days of age.	Yoshioka et al., 1983
Pig	DGGE RT-PCR	Ascending colon	Sow-reared piglets had increased concentrations of bifidobacteria. Formula-fed piglets had increased concentrations of *Clostridium* cluster IV and XIVa and *Bacteoroides vulgates.*	Li et al., 2012
Pig	454 GS-FLX Titanium pyrosequencing	Cecum	Sow-reared piglets were dominant in *Prevotella, Oscillibacter,* and *Clostridium.* Sow-reared piglets had increased expression of galactose mutarotase (metabolizes lactose) and transketolase (phage resistance, LPS metabolism, and flagellar motor function). Formula-fed piglets had increased concentrations of *Bacteroides, Parabacteroides,* and *Alistipes* and increased transcript utilization of L-arabinose and the sugar alcohol mannitol .	Porokyo et al., 2010, 2011

Table 2. Effect of high fat diet on gut microbiota.

Source	**Type**	**Feeding Length**	**Site**	**Detection Method**	**Findings**	**References**
Mice C57BL/6J	Unknown	4 wks	Feces	Pyrosequencing	-Increase Erysiphelotrichi and Bacilli - Decrease in Bacteroidetes	Turnbaugh et al., 2009b
Mice C57BL/6J	Unknown	8 wks	Cecum	Pyrosequencing	-Increase in Firmicutes, mollicutes -Decrease in Bacteroidetes	Turnbaugh et al., 2008
Mice C57BL/6J	Safflower Oil	21 wks	Feces Colon	Pyrosequencing	-Increases in Clostidiaceae, Delta-Proteobacteria, Desulfovibrionaceae, and Mollicutes. -Decreased Bacteroidaceae, Prevotellaceae, and Rickenellaceae	Hildebrandt et al., 2009
Rats Sprague-Dawley	36.3% Saturated FA 45.3%MUFA 18.5% PUFA	8 or 12 wks	Cecum	RT-PCR	-Increases in Bacteroidetes, Clostidiales -Decreases in Firmicutes -Increases intestinal permeability and inflammation by activating TLR-4	De La Serre et al., 2010
Mice C57BL/6J	Corn Oil Lard	2 or 4 wks	Cecum	FISH DGGE	-Increased gram-negative bacteria increases LPS in the intestine and endotoxins -Increases intestinal permeability - Decreased gram-positive bacteria -Decreased Bacteroidetes, bifidobacteria .	Cani et al., 2007, 2008
Humans	Unknown	8 wks	Feces	Cultured	-Decreased Bifidobacteria	Brinkworth et al., 2009
Humans	Unknown	Unknown	Feces	Pyrosequencing	-Increased in Bacteroidetes, especially *Bacteroides*, Actinobacteria -Decreased in Firmicutes, Proteobacteria	Wu et al., 2011
Mice C57BL/6J	Saturated milk fat or PUFA	24 wks	Cecum	Pyrosequencing	-Increased Bacteroidetes - Milk fat increased the pathogen Bilophila wadsworthia, which flourishes in the presence of the taurine-conjugated bile acid -Decreasesd Firmicutes	Devkota et al., 2012
Mice C57BL/6J	Soybean oil Lard	4 wks	Cecum	DGGE qPCR	-Decrease in total bacteria	Dewulf et al., 2011

Table 3. Effect of carbohydrates on gut microbiota

Fiber	Source	Type	Findings	References
Prebiotic	*In vitro*	Inulin and FOS	-Bifidogenic effects	Van de Wiele et al., 2007
	Humans	Inulin and FOS	-Bifidogenic effects	Gibson et al., 1995; Bouhnik et al., 1999,2007; Rao, 2001; Touhy et al., 2001; Kruse et al., 1999; Brighenti et al., 1999
	Rats	Inulin and FOS	-Bifidogenic effects	Van der Abbeele et al., 2011
	Pigs	Inulin and FOS	-Did not change GM	Mikkelsen et al., 2003; Mikkelsen and Jensen, 2004; Janczyk et al., 2010
	Pigs	Inulin and FOS	-Increased lactic acid bacteria	Lui et al., 2012
	Humans and *in vitro*	GOS	-Increased the concentration of bifidobacteria	Walton et al., 2012
	Pigs	GOS	-No differences in GM	Mikkelsen et al., 2003; Tsukahara et al., 2010
	In vitro	RS	-Increases in *Bifidobacterium* and *Atopobium* spp	Lesmes et al., 2008
	Rats	RS	-Increases the growth of bifidobacteria	Kleesen et al., 1997; Silvi et al., 1999; Wang et al., 2002; Conlon and Bird, 2009; Young et al., 2012
	Rats	RS Type 2	-Decreased Bacteroides -Increases total culturable bacteria, lactobacilli, streptococci, and enterobacteria	Kleessen et al., 1997
	Rats	RS	-Increase in lactobacilli and bifidobacteria in the cecum	Silvi et al., 1999
	Aged mouse	RS Type 2	-Increased Bacteroidetes due to unclassified members of the phylum	Tachon et al., 2012
	Rats	RS	-Increases in Bacteroidetes and Actinobacteria due to the growth of Porphyromanadaceae and Bifidobacterium, respectfully, -Decreases in Firmicutes due to clostridium	Young et al., 2012
	Humans	RS	-Decreased Firmicutes -Increased Bacteroidetes and Actinobacteria -Increase in Ruminoccocus	Martinez et al., 2010 Abell et al., 2008
	Pigs	RS	-No changes in GM	Castillo et al. (2007)
	Pigs	RS	-Increase in Lactobacillus	Metzler et al. (2009)

Other	Pigs	Wheat middlings, sunflower seeds, potato starch, and SBP	-Increased lactobacilli -No change in *E. coli*	Bikker et al., 2006; 2007
	Pigs	Wheat bran and SBP	-No change in Lactobacilli -Increased bacteroides, *Clostridium leptum* and *C. coccoides*	Pieper et al., 2012
	Pigs	Soybean hulls and SBP	-Decreased aerobic mesophilic bacteria, anerobic sulfite-reducing bacteria, and *Enterococcus*	Montagne et al., 2012
	Pigs	SBP	-Decreased *Clostridia*	Jeaurond et al., 2008
	Pigs	SBP	-No change in GM	Castillo et al., 2007
	Pigs	SBP and FOS	-Increase in *Ruminococcus*	Konstantinov et al., (2003)
	Pigs	Inulin, lactulose, wheat starch and SBP	-Increased *Lactobacillus reuteri* and *L. amylovorus*	Konstantinov et al., 2004
	Pigs	Fenugreek seed	-Increase *Lactobacillus* and clostridium cluster I -Decreased *Eschieria, Hafnia*, and *Shigella*	Zentek et al., 2012
Simple Sugars	Pigs	Lactose	-Increased growth lactobacillus	Pollman et al., 1980
	Pigs	High (215 g lactose/kg) versus low levels of lactose (125 g lactose/kg)	-High levels of lactose increased *Bifidobacteria* populations	Pierce et al., 2007
	Pigs	Lactose	-Increased *L. johnsonii*, and *L. reuteri* -Decreased *L. delbrueckii*	Tran et al., 2012
	Humans digesters and maldigesters	Lactose	-No changes in GM	Szilagyi et al., 2009
	Humans digesters and maldigesters	25 g of lactose twice a day	-Maldigesters bifidobacteria counts increased, with no change in lactobacilli counts	Szilagyi et al., 2010
	Infants with allergies to cow's milk	Lactose	-Increased bifidobactia and lactic acid bacteria -Decreased bacteroides/clostridia	Francavilla et al., 2012

Table 4. Effect of protein on gut microbiota

Source	**Type**	**Findings**	**References**
Pigs	Increasing levels of CP	Increased *E. coli* Decreased *Lactobacilli* and *Bifidobacteria*	Pierce et al., 2007
Adult cats	High protein	Increased *C. perfringens* Decreased *Bifidobacteria*	Lubbs et al., 2009
Kittens	High protein	Decreased *Bifidobacteria, Lactobacillus* and *E. coli* amounts in fecal samples Increased *Clostridium, Faecalibacterium, Ruminococcus, Blautia,* and *Eubacterium* Decreased *Dialister, Acidominococcus, Bifidobacteria, Magasphaera,* and *Mitsuokella*	Vester et al., 2009 Hooda et al., 2012
Pig	Glycoproteins	Decrease the incidence of *E. coli* adhesion	Rhoades et al., 2005; Hermes et al., 2011; 2012
In vitro Pig	Glycoproteins	Increase *Bifidobacteria* and *Lactobacillus*	Idota et al., 1994; Bouhallab et al., 1994 Hermes et al., 2012

Chapter 2

Effect of Dam Parity on Progeny Health Status and Growth Performance

INTRODUCTION

Pig health is a major concern for pork producers as it has a large impact on production costs as well as animal well being. Several factors can influence pig health, such as the environment, genetics, passive immunity, and age of the dam. The age of the dam impacts pig health through several factors, including providing adequate nutrients and passive immunity in colostrum and milk, therefore affecting growth performance. It has been hypothesized that first parity progeny have a decreased health status compared to older parity dams possibly due to decreased passive immunity provided by gilts compared to that provided by multiparous sows.

DAM PARITY DIFFERENCES

Parity affects the concentration of nutrients and other components in sow colostrum and milk. Growth factors in milk are affected by parity. For example, IGF-I concentrations were decreased in gilts compared to sows (Averette et al., 1999). The development of the small intestine is greatly affected by IGF-I; therefore impacting gut closure. If a piglet's gut does not develop fast enough there is a greater probability that it will remain susceptible to pathogenic microbes, thereby increasing the probability of morbidity and mortality. In addition, the concentration of α-tocopherol, an essential source of vitamin E, in milk from colostrum to 21 d was different between primiparous and multiparous sows, as α-tocopherol levels in colostrum increased from P1 to P3 then declined through P5 (Mahan et al., 2000); however, no differences were found in P2 through P5 milk samples.

Dam parities effect on litter and growth performance has been well documented. The ADFI of P1 gilts is lower through gestation and lactation compared to multiparous sows, with the greatest differences existing between P1 and P2 dams (Mahan, 1998). First parity dams have fewer live pigs per litter (Mahan, 1991, 1994, 1998; Averette et al., 1999; Peters and Mahan, 2010; Smits et al., 2011), smaller litter weights (Hendrix et al., 1978 Wilson and Johnson, 1980; Mahan, 1994; Mahan et al., 2000; Peters and Mahan, 2010) and decreased pig gain (Hendrix et al., 1978; Mahan, 1991, 1998; Kemme et al., 1997; Averette et al., 1999; Peters and Mahan, 2008; Smits et al., 2011), as well as fewer stillbirths, mummies, and mortality (Mahan, 1994; Averette et al., 1999). Previously, we compared P1 dams to P4 dams and observed no differences in litter size; however, P4 progeny had larger piglet weights throughout lactation (Carney-Hinkle, et al., 2012). Research by Mahan (1994, 1998) and others (Mahan et al., 2000) indicates that number of pigs born live and pig weights may peak at P3, then decline into subsequent parities. This would explain why no differences were observed in litter size among P1 and P4 dams.

Growth performance of progeny is also affected by dam parity. Weaning weights of P1 piglets on d 21 are decreased compared to progeny derived from P2 or greater dams (Wilson and Johnson, 1980; Wood et al., 1990; Culbertson et al., 1997; Mahan, 1998). Average daily gain of P1 piglets is decreased compared to piglets derived increasing parity (Hendrix et al., 1978; Mahan, 1991, 1998; Kemme et al., 1997; Averette et al., 1999; Peters and Mahan, 2008; Smits et al., 2011). Previously, we observed parity 4 progeny were heavier at birth and weaning. This increase in growth performance may be due to increased health performance of pigs.

Health status could be affected by the concentration of antibodies provided by transfer of passive immunity from the dams' colostrum. A major component of passive immunity is immunoglobulins (Ig), in particular IgG and IgA (Rooke and Bland, 2002). As neonates, piglets cannot synthesize their own antibodies, so the level of immunological protection is determined by the amount the piglet receives from its mother through colostrum and milk. The transfer of passive immunity is essential for piglet survival (Hendrix et al., 1978; Rooke and Bland, 2002). The concentration of IgG is highest in colostrum which then decreases significantly after 24 h, while IgA is high in colostrum, but remains the most abundant Ig in milk (Klobasa et al., 1987; Carney-Hinkle et al., 2012).

Immunoglobulin concentrations in sow serum increase with age. The concentration of dam circulating Ig is important, as Ig in serum in transported to the dams colostrum and milk. The transport of Ig to the mammary gland is different for IgG and IgA. The transfer of IgG is transported from dam serum, while IgA is derived from plasma cells that were transported to the mammary gland from the gut associated lymphoid tissues (GALT; Wagstrom et al., 2000; Wheeler et al., 2007). Dam serum concentrations of IgG are generally high until d 90 of gestation, at which time IgG moves to the mammary gland, so concentrations at d 114 of gestation and d 0 of lactation are much lower (Klobasa et al., 1985 a,b). Dam parity influences dam serum Ig concentrations as they can increase with increasing parity (Klobasa et al., 1985a; Klobasa et al., 1986), however we previously reported no differences between P1 and P4 dams (Carney-Hinkle et al., 2012).

It would be expected that differences in dam serum Ig concentrations would be reflected in dam colostrum and milk concentrations, however, mixed results have been reported. Inoue (1981) and others (1980) found that parity was the most influential effect on IgA and IgG concentrations in colostrum as concentrations were low in P1-P3 dams, but higher in ≥ P4 dams. When we compared P1 and P4 dams, we detected a tendency for IgA concentrations to be higher in P4 dams with no differences in IgG concentrations (Carney-Hinkle et al., 2012). Klobasa et al. (1987) found that parity affected Ig concentrations, but could not define an increase or decrease based on parity number. Reasons for these discrepancies between the two studies could be due to newer methods of Ig quantification and how colostrum/milk was collected as concentrations can vary from upper and lower mammary glands.

Despite the discrepancies in dam colostrum and milk concentrations, differences are almost always observed between progeny of different dam parity. Results by Klobasa et al. (1986) observed first parity progeny had decreased serum IgG, IgA, and IgM concentrations compared to the progeny of multiparous sows. The researchers observed dam parity effects for IgG in sows and piglet serum, with no differences observed in milk whey concentrations. Preliminary data collected during lactation resulted in increased circulating serum IgA and IgG concentrations in the progeny of P3 sows compared to the progeny of P1 gilts (Burkey et al., 2008). The differences in colostrum/milk concentrations and piglet serum between parities could be due to an increase in milk yield of P3 dams (Devillers et al., 2007). Other immune components are also affected by parity. Lymphocyte and macrophage concentrations in sow milk are decreased in primiparous sows compared to multiparous sows (Hurley and Grieve, 1988). These differences among dam parity are possibly affecting the health status of progeny derived from different dam parity in subsequent phases of production (nursery and finishing).

Differences in transfer of passive immunity may affect gut microbial communities. Gut microbiota have been shown to play a role in immune system development. The immune system must learn early in its development which microbes, that colonize the gastrointestinal system, are commensal versus pathogenic. Once the immune system has developed, it should know which bacteria are helpful versus harmful. Thus, the initial bacteria to colonize in the gastrointestinal tract influence the immune systems performance long after its initial development (Hooper et al., 2012). Comparisons of gut microbial communities between parity 1 and parity 4 progeny using denaturing gradient gel electrophoresis showed increased diversity of parity 1 progeny compared to parity 4 progeny (Carney et al., 2009b). A more detailed look at bacterial communities is necessary to determine possible parity differences.

Little is known with respect to the effects of dam parity on nursery pig health and performance. However, is has been observed that in parity segregated commercial systems, P1 progeny need more intensive care and management. Results from a parity segregated farm show that the cost of medications provided to nursery pigs was greater than that expended on progeny derived from parity 2 and greater progeny (Moore, 2001). Mortality of nursery pigs was greater for primiparous progeny compared to multiparous progeny. The same trend continued into the finisher phases with P1 progeny having greater medication costs and greater mortality rates than progeny derived from multiparous sows (Moore, 2001). More information is needed to determine how parity effects litter health status and growth performance. Previously, we observed greater BW and ADFI in P4 pigs compared to P1 pigs (Carney et al., 2009a). While the passive immunity of progeny in the nursery period declines rapidly after weaning, differences in Ig concentration have been observed. In a previous study comparing P4 pigs to P1 pigs a significant parity by dietary treatment interaction was observed as P1 pigs provided antibiotics and P4 pigs on an antibiotic free diet had the lowest circulating IgA concentrations. More research is needed to understand the interactions between passive immunity and antibiotic use.

CONCLUSION

In conclusion, dam parity affects nutrient composition and passive immunity of colostrum and milk as well as dam and progeny performance and the gut microbial community. The greatest differences between parities are observed between P1 and ≥P2 dams. These difference observed between dam parity could affect pig health performance in the nursery and finishing periods.

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Chapter 3

Effect of dam parity on litter performance, passive immunity, and fecal microbial populations (parity 1 vs. 3)

ABSTRACT: Previous research has shown that parity (P) 4 progeny have greater weaning weights and decreased microbial diversity. The objective of this experiment was to evaluate litter performance, passive immunity, and fecal microbiota among P1 (n = 56) and P3 (n = 49) dams and their progeny. Blood samples were collected from P1 and P3 dams on d 90 and 114 of gestation and d 0 of lactation and from their progeny (n = 4 pigs/litter) on d 0, 7, and 14 of lactation. Colostrum/milk samples were collected from dams on d 0, 7, and 14 of lactation. Fecal samples were collected from dams (d 7) and their progeny (n = 4 pigs/litter; d 0, 7, and 14 of lactation). Serum and milk samples were analyzed for immunoglobulin (Ig; G and A) concentrations via ELISA. Microbial fingerprinting of fecal samples from dams and progeny were performed via DGGE. No differences were observed between parities in total born, live born, mummies, deaths, or total weaned. A greater number of stillborns ($P < 0.013$) were observed for P3 dams compared to P1 dams (1.0 and 0.5 Pigs, respectively). Progeny BW was increased for P3 litters on d 0, 7, 14, and at weaning (d 16) compared to P1 dams ($P < 0.001$; 14.6 and 18.4 kg, 23.0 and 28.9 kg, 38.9 and 48.0 kg, 43.8 and 57.2 kg for P1 and P3 by day, respectively). Dam serum IgG concentrations on d 114 of gestation were increased ($P < 0.001$) for P3 dams compared to P1 dams. Parity × day interactions were observed for progeny IgG ($P < 0.01$) and IgA ($P < 0.001$) concentrations. Progeny derived from P3 dams had greater concentrations IgG on d 0 and 14 compared to P1 progeny, and IgA concentrations were greater in P3 progeny on d 0. There were no effects of dam parity on fecal microbial fingerprinting (diversity or similarity indices) in samples obtained from dams or their progeny ($P > 0.50$). Litter performance and transfer of passive immunity are affected by dam parity.

Key words: dam parity, gut microbiota, passive immunity

INTRODUCTION

The transfer of passive immunity is essential for new-born piglet survival (Rooke and Bland, 2002). The main source of passive immunity is Immunoglobulins (Ig), especially IgG and IgA, transferred through colostrum to progeny the first day after birth. Multiparous sows transfer higher concentrations of IgG and IgA to their progeny than primiparous sows (Klobasa et al., 1986). The differences in passive immunity between parities may affect the development of the immune system and gut microbiota. A very complex interaction between the immune system and commensal gut bacteria exists, as the immune system controls the gut microbiota, while the gut microbiota influence immune development (Hooper et al., 2012; Clemente et al., 2012). Differences in gut microbiota in the early stages of pig development may affect piglet health status later on in life, especially when faced with disease.

Previous research conducted at the University of Nebraska – Lincoln has indicated that growth performance, transfer of passive immunity, and gastrointestinal microbial populations may be affected by dam parity. Parity (P) 4 progeny were heavier at birth and weaning, and had increased microbial species richness (number of species and abundance) compared to P1 progeny (Carney-Hinkle et al., 2012). However, we were unable to detect any differences in passive immunity. Mahan (1991, 1994, and 1998) has reported that dams may reach a peak in litter performance at P3, then decline slightly at P4. Passive immunity may be affected by this peak. So, based on our previous research, we hypothesis that litter performance, passive immunity, and fecal microbiota will be affected by dam parity. In particular, we expect greater differences between P3 and P1 dams and progeny than were found between P4 and P1 dams and progeny. Therefore, the objective of this study was to evaluate litter performance, passive transfer of immunity, and fecal microbiota between P1 and P3 dams and progeny.

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 (n = 56) and P3 dams (n = 49) that farrowed during a 19-d period beginning September 11, 2009, and ending September 30, 2009. All P1 and P3 dams that farrowed in this time period were included in the experiment. Dams and pigs used in this experiment were of a high health status with no positive tests for porcine reproductive and respiratory syndrome virus. Dams were co-mingled and housed in stalls during gestation and moved to farrowing crates approximately 5 d prior to their expected farrowing date. Fostering of piglets occurred within parities to equalize litter size. Dam and litter performance parameters recorded included: Number of pigs/litter (total born, born live, stillbirths, mummified fetuses, pigs weaned, and pre-weaning mortality), progeny weight at birth (BW) and weaning (WW). On d 0 (following parturition between 0600 and 1000 h) all piglets were processed including injection of 200 mg Fe (Fe dextran, Uniferon 200, Watchung, NJ), tail-docking, and ear-tagging. No antibiotics were administered during the current experiment. Boar piglets were castrated before 3 d of age. All piglets from each litter were weighed on d 0, 7, 14, and at weaning (16 ± 0.3 d of age). The entire experiment was conducted at the University of Nebraska Agricultural Research and Developmental Center Swine Unit near Mead, NE.

Blood, milk, and fecal sample collection

All blood, milk, and fecal samples were collected between 0600 and 1200 h. Blood samples were collected from all sows via jugular venipuncture at 2 time points during gestation (d 90 and 114) and immediately following parturition (d 0). During lactation, on d 0, 7, and 14 colostrum/milk samples were collected by hand from all functional mammary glands (5-15 mL per dam) in a sterile tube and frozen (-20^{o}C) for subsequent analyses. For milk samples, 2 mL of oxytocin (Oxytocin-RXV, Bimeda-MTE-Animal Health, Inc., Cambridge, Canada) was administered, intramuscularly near the vulva, to facilitate milk collection.

Blood samples were collected from 4 randomly selected piglets from each litter via jugular venipuncture on d 0, 7, and 14. Piglets were randomly selected for sampling on d 0, then assigned eartags to identify same pigs on d 7 and 14. Dam and piglet serum were harvested by centrifugation (1,500 × *g* for 20 min at 4°C) and frozen (-20˚C) for subsequent analyses.

Fecal samples were collected from all dams on d 7 of lactation and 4 previously randomly selected progeny per dam on d 0, 7, and 14 of lactation. Antibiotics were not given to any sows or piglets sampled. Fecal samples were collected directly from the rectum using a fecal loop (KV Supply, David City, NE) on d 0, 7, and 14 from 4 piglets from each litter. Fecal samples were stored in PBS and frozen (-20˚C) for further analyses (described below).

Milk and serum analyses

Colostrum and milk samples were diluted (1:50,000) and concentrations of IgA and IgG were quantified as described below. Dam and piglet serum samples were diluted for IgG and IgA analyses (1:100,000; 1:25,000 for d 0 and 7 progeny and 1;1,000 for dams d 14 progney samples, respectfully). Concentrations of IgA and IgG in serum, colostrum, and milk were quantified via swine-specific enzyme-linked immunosorbent assays (ELISA; Bethyl Labs Inc., Montogomery Tx.) using goat anti-pig antibody. The range of the Ig ELISAs was 7.81 to 1,000 ng/mL; sensitivity was 2.0 ng/mL. The intra- and interassay CV for the IgA ELISAs was 7.3 and 22.1% for serum and milk analysis. The intra- and interassay CV for the IgG ELISAs was 8.4% and 23.9%, for serum and milk analyses.

Fecal Microbial DNA Extraction

Due to limited gel capacity and heavy gel bias 12 dams per parity and 1 piglet per litter were randomly selected for fecal microbial analysis. The same piglet was used for analysis of d 0, 7, and 14 fecal microbial analyses. Extraction of DNA from all fecal samples was carried out according to the methods described by Martinez et al. (2009). Briefly, fecal samples were thawed and a 500 mL aliquot of each sample was used for DNA isolation. All samples were washed with PBS and centrifuged (8,000 × *g* for 5 min); the wash step was repeated 3 times. The bacterial pellet was resuspended in 750 μL Lysis buffer (200 m*M* NaCl, 100 m*M* Tris [pH 8.0]), 20 m*M* EDTA, 20 mg/mL Lysozyme) and transferred to a microcentrifuge tube containing 300 mg of 0.1 mm zirconium beads (BioSpec Products; Bartlesville, OK). Samples were incubated for 20 min at 37˚C. After incubation, 85 μL of 10% SDS solution and 40 μL Proteinase K

(15mg/mL; Sigma; St. Louis, MO) were added to samples followed by another incubation period at 60˚C for 15 min. Phenol:chloroform:isoamyl alcohol (25:24:1, 500 µL) was added and the samples were homogenized in a MiniBeadbeater-8 (BioSpec Products) at maximum speed for 2 min. Samples were placed on ice before separating layers by centrifugation at 10,000 × *g* for 5 min. The top layer was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1) and twice with chlorophorm:isoamyl alcohol; DNA was recovered by standard ethanol precipitation. Pellets were dried for 30 min at room temperature. The DNA pellet was resuspended in 100 µL of Tris/HCl Buffer (10 m*M*, pH 8.0). The DNA samples were stored at -20˚C for subsequent analyses.

Denaturing gradient gel electrophoresis (DGGE)

Polymerase chain reaction procedures were performed on the extracted DNA using bacterial specific primers to the conserved regions flanking the variable V3 region of 16s rDNA. Each PCR mixture contained 20 pmol of primers PRBA338fGC (5'CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG 143 ACTCCTACGGGAGGCAGCAG'3) and PRUN518r (5'-ATTACCGCGGCTGCTGG -3'). Denaturing gradient gel electrophoresis was performed as described by Walter et al. (2000) using a DCode universal mutation detection system (Bio-Rad, Hercules, USA). Visualization of the DNA bands in the DGGE gels was carried out by standard ethidium bromide staining and photographed using the InGenius gel documentation system (Syngene, Frederick, MD). The DGGE images were analyzed using BioNumerics software Version 5.0 (Applied Maths, Kortrijk, Belgium). Distance matrices were generated by manually assigning bands and normalizing banding patterns used to generate distance matrices. Matrices were used to calculate the Pearson product moment correlation coefficient for all pair-wise combinations of patterns. Pair-wise combinations compare profiles based on the entire densitometric curve; therefore, accounting for both band position and intensity. Using the BioNumerics software, 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 similarity of the gut microbiota within parity, individual piglet DGGE profiles were compared by Pearson's pair-wise comparison, thus obtaining similarity coefficients.

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.

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

$$\text{Simpson's coefficient} = \sum_{i=1}^{n} \left((ni*(ni\text{-}1))/(N*(N\text{-}1))\right)$$

Statistical Analysis

All data were analyzed as a randomized complete design using dam as the experimental unit. Analysis of variance was performed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Litter performance and progeny birth weight and weaning weight included effect of parity in the model with body weight and weaning weight including a random statement to ensure dam was the experimental unit. Dam serum and colostrum/milk included parity, day, and their interaction in the model with day considered a repeated measure. Progeny serum Ig concentrations were analyzed for parity, day, and their interaction with day as a repeated measure and random statement included to ensure dam was the experimental unit. Diversity and similarity microbial indexes were analyzed for effects of parity, day, and their interaction. Least squares means were calculated for each independent variable.

RESULTS

Dam and litter performance are presented in Table 1. No differences were observed between parities in total born, live born, mummies, deaths, or total weaned; however, P3 dams had a greater number of stillborns ($P < 0.013$) compared to P1 dams

(1.0 and 0.5 pigs, respectively). Progeny body weight was increased for P3 progeny on d 0, 7, 14, and at weaning (d 16) compared to progeny from P1 dams ($P < 0.001$).

Serum IgA and IgG concentrations for P1 and P3 dams are presented in Figure 1. A significant parity × day interaction ($P < 0.022$) was observed for dam serum IgG concentrations (Figure 1). On d 90, IgG concentrations in P3 dams were greater ($P < 0.05$) compared to P1 dams. No differences between dam parity found on d114. Parity 1 dams on d 0 had the lowest IgG concentrations. No effects of dam parity were observed for circulating IgA in dams ($P = 0.56$). However, as expected, a significant day effect was observed as circulating IgA concentrations were greater ($P < 0.05$) on d 114 compared to all other timepoints.

The concentration of Ig (A and G) in milk samples during lactation (d 0, 7, and 14) were not affected by dam parity ($P > 0.40$; Figure 2). However, a significant effect of day was observed where greater ($P < 0.001$) concentrations of Ig (A and G) were observed on d 0 compared to all other timepoints when means were averaged among both parities.

Parity × day interactions were observed for progeny IgG ($P < 0.001$) and IgA ($P < 0.001$) serum concentrations (Figure 3). The concentration of Ig (A and G) in serum from P1 and P3 progeny were greater ($P < 0.001$) on d 0 compared to all other timepoints. In addition, P3 progeny had greater ($P < 0.001$) concentrations of Ig (A and G) on d 0, and greater ($P = 0.041$) concentrations of IgG on d 14 compared to P1 progreny.

Denature gradient gel electrophoresis was used to evaluate the microbial fingerprint of dam and progeny feces. 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. 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. There were no effects of dam parity on fecal microbial fingerprinting (diversity or similarity indices) in samples obtained from dams or their progeny ($P > 0.50$).

DISCUSSION

We conducted this experiment to determine if dam parity affected litter and growth performance, passive transfer, and fecal microbial ecology by comparing P1 and P3 dams and their progeny. Litter and growth performance and passive transfer were increased with P3 dams and progeny compared to P1 progeny. However, dam parity did not affect fecal microbiota.

Dam parities effect on litter and growth performance has been well documented. First parity dams have less live pigs per litter (Mahan, 1991, 1994, 1998; Averette et al., 1999; Peters and Mahan, 2010; Smits et al., 2011), smaller litter weights (Hendrix et al., 1978 Wilson and Johnson, 1980; Mahan, 1994; Mahan et al., 2000; Peters and Mahan, 2010) and decreased pig gain (Hendrix et al., 1978; Mahan, 1991, 1998; Kemme et al., 1997; Averette et al., 1999; Peters and Mahan, 2008; Smits et al., 2011), as well as fewer stillbirths, mummies, and mortality (Mahan, 1994; Averette et al., 1999). In a previous experiment comparing P1 dams to P4 dams, no differences were found in litter size, however, P4 progeny had larger piglet weights throughout lactation (Carney-Hinkle, et al., 2012). Research by Mahan (1994, 1998) and others (Mahan et al., 2000) indicates that number of pigs born live and pig weights may peak at P3, then decline into subsequent parities. Therefore, for this experiment, larger differences in litter performance were expected between P1 and P3 dams. As expected we did see increased stillbirths and increased piglet body weight with P3 dams compared to P1 dams, however no differences in litter size were observed.

The transfer of passive immunity is essential for piglet survival (Hendrix et al., 1978; Rooke and Bland, 2002). A major component of passive immunity is immunoglobulins, in particular IgG and IgA (Rooke and Bland, 2002). The concentration of IgG is highest in colostrum which then decreases significantly after 24 h, while IgA is high in colostrum, but remains the most abundant Ig in milk (Klobasa et al., 1987; Carney-Hinkle et al., 2012). This study followed these trends as IgG concentrations were highest in colostrum (d 0) and IgA concentrations were highest in milk (d 7 and 14). Parity did not have an effect on colostrum/milk IgA or IgG concentrations, which is inconsistent with previous reports. Inoue (1981) and others (1980) found that parity was the most influential effect on IgA and IgG concentrations in colostrum as concentrations were low in P1-P3 dams, but higher in ≥ P4 dams. When we compared P1 and P4 dams we detected only a tendancy for IgA concentrations to be higher in P4 dams with no differences in IgG concentrations (Carney-Hinkle et al., 2012).

Klobasa et al. (1987) found that parity affected Ig concentrations, but could not define an increase or decrease based on parity number. Reasons for these discrepancies could be due to newer methods of Ig quantification and how colostrum/milk was collected as concentrations can vary from upper and lower mammary glands.

The transport of Ig to the mammary gland is different for IgG and IgA. The movement of IgG to colostrum is hormonally regulated and dervived from dam serum (Wagstrom et al., 2000; Wheeler et al., 2007). Plasma cells that were transported to the mammary gland from the gut associated lymphoid tissues (GALT) produce IgA in dam colostrum and milk (Wheeler et al., 2007). Dam serum concentrations of IgG are generally high until d 90 of gestation, at which time IgG moves to the mammary gland, so concentrations at d 114 of gestation and d 0 of lactation are much lower (Klobasa et al., 1985 a,b), which is consistent with results obtained in the current study. Dam parity influences dam serum Ig concentrations as concentrations can increase with increasing parity (Klobasa et al., 1985a; Klobasa et al., 1986), however we previously reported no differences between P1 and P4 dams (Carney-Hinkle et al., 2012). In the current study, P3 dams had higher concentrations of IgG on d 90 of gestation and d 0 of lactation than P1 dams. Unfortunately this difference in dam serum Ig concentrations was not reflected in colostrum and milk concentrations.

Despite finding no differences in sow colostrum and milk Ig concentrations between dam parities, P3 progeny had higher concentrations of IgG and IgA compared to P1 progeny. This is very similar to previous results (Klobasa et el. 1986), which compared first parity to multiparous sows. The researchers observed dam parity effects for IgG in sows and piglet serum, with no differences observed in milk whey concentrations. The differences in colostrum/milk concentrations and piglet serum between parities could be due to an increase in milk yield of P3 dams (Devillers et al., 2007).

A complex interaction between the immune system and commensal gut bacteria has been established. The immune system has regulatory mechanisms which can control the development of gut microbiota and determine commensal bacteria from pathogenic bacteria (Hooper et al., 2012). Meanwhile, commensal bacteria play a crucial role in the development of the immune system, as well as disease development, including obesity, type I diabetes, metabolic syndrome, autoimmune disorders and allergic responses (Hooper et al., 2012; Clemente et al., 2012). We therefore, wanted to determine if differences in passive immunity between dam parity could affect the gut microbiota using DGGE technology.

Previously we found that P4 progeny had decreased species richness compared to P1 progeny on d 7 of lactation, as well as P1 progeny having a more similar bacterial community among the P1 population compared P3 progeny (Carney-Hinkle et al., 2012). No differences in species richness, evenness, or community similarity between dam parity were found when we compared P1 dams and progeny to P3 dams and progeny. Reasons for not finding differences in P1 and P3 dams and progeny could be due to an absence of detail in DGGE technology, as rare bacterial communities are not detectable.

In conclusion, litter performance and transfer of passive immunity may be affected by dam parity. The level of passive immunity acquired may directly affect the development of active immunity and indirectly affect the health and performance of the piglet. The results described in this report suggest that mature dams (≥ P3) provide their progeny with advantages in transfer of passive immunity.

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Table 1. Treatment effects of sow parity on litter and pig measurements.

	Parity				
Variable	1	SEM	3	SEM	*P* - value
No. of Sows	56		49		
Gestation Length	113.5	0.16	115	0.17	< 0.001
No. of Pigs per litter					
Total born	12.57	0.55	13.26	0.59	0.390
Born live	11.66	0.50	11.89	0.53	0.747
Stillbirths	0.50	0.14	1.00	0.14	0.013
Mummified fetuses	0.41	0.09	0.36	0.10	0.748
Mortality (pre-weaning)	1.91	0.23	2.14	0.25	0.496
Weaned	9.57	0.39	9.73	0.42	0.777
Piglet BW, kg					
Birth	1.22	0.03	1.53	0.03	< 0.001
d 7	2.25	0.05	2.83	0.06	< 0.001
d 14	3.81	0.09	4.90	0.09	< 0.001
Weaning (d 16)	5.24	0.28	6.21	0.28	0.016

Table 2. The effect of dam parity (P1 vs. P3) on diversity indexes[1] of microbial populations in piglets (1 pig per litter, 12 litters per parity).

	Diversity Coefficients				
	Shannon's				
	Parity 1	SEM	Parity 3	SEM	*P*-Value
Dams	2.09	0.19	2.14	0.19	0.845
Progeny					
d 7	1.02	0.28	1.29	0.28	0.772
d 14	1.20	0.80	1.03	0.29	
	Simpson's				
	Parity 1	SEM	Parity 3	SEM	*P*-Value
Dams	0.18	0.03	0.17	0.03	0.699
Progeny					
d 7	0.27	0.05	0.22	0.06	0.854
d 14	0.23	0.05	0.26	0.06	

[1]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 greater diversity.

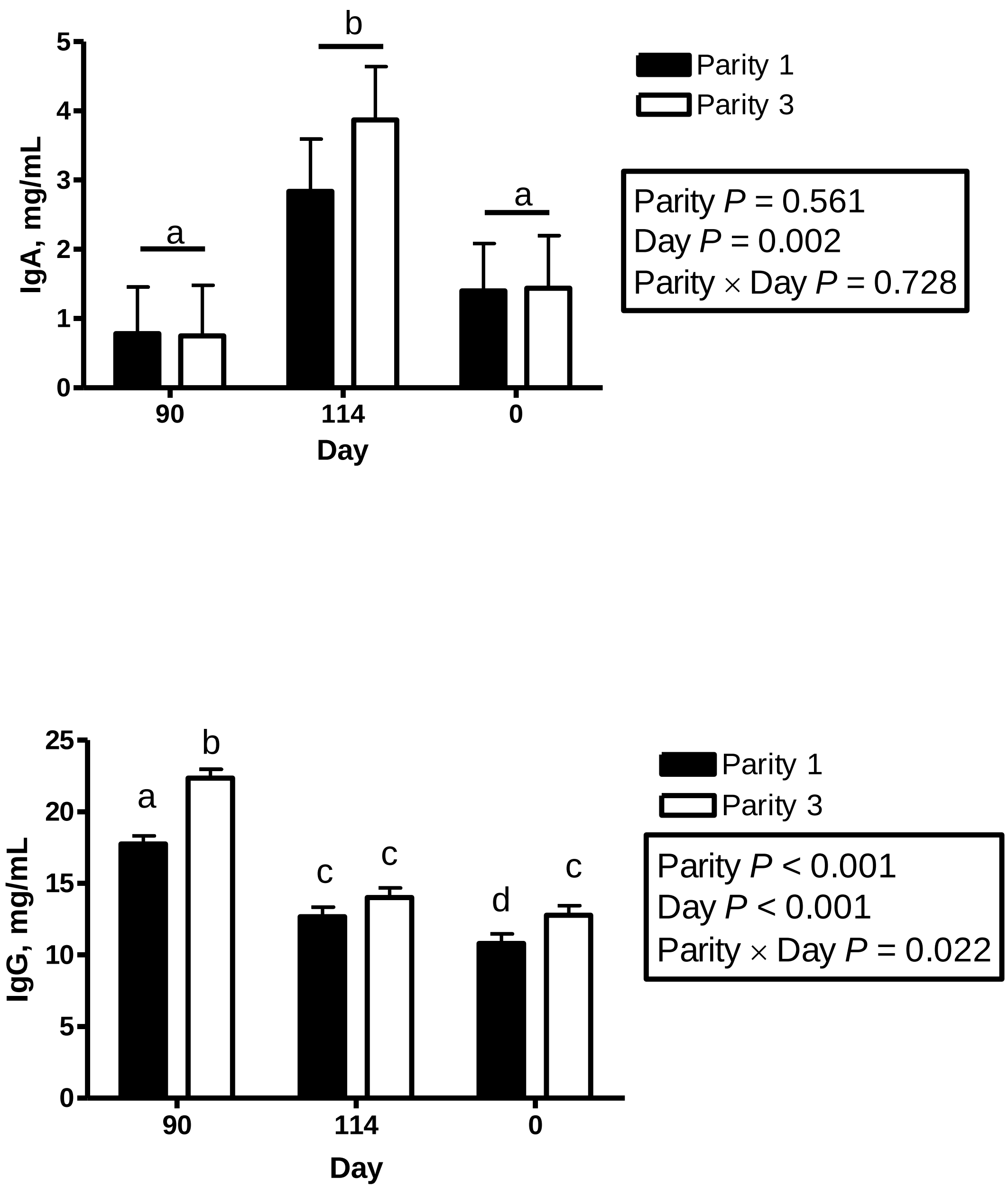


Figure 1. Circulating concentrations of IgA (top panel) and IgG (bottom panel) in parity (P) 1 and P3 dams. Immunoglobulin concentrations were evaluated in serum obtained at d 90 and 114 of gestation and immediately following parturition (d 0). Each bar represents the least-squares mean (± SEM) of 56 and 49 observations for P1 and P3 dams, respectively. Bars without a common letter differ significantly ($P < 0.05$).

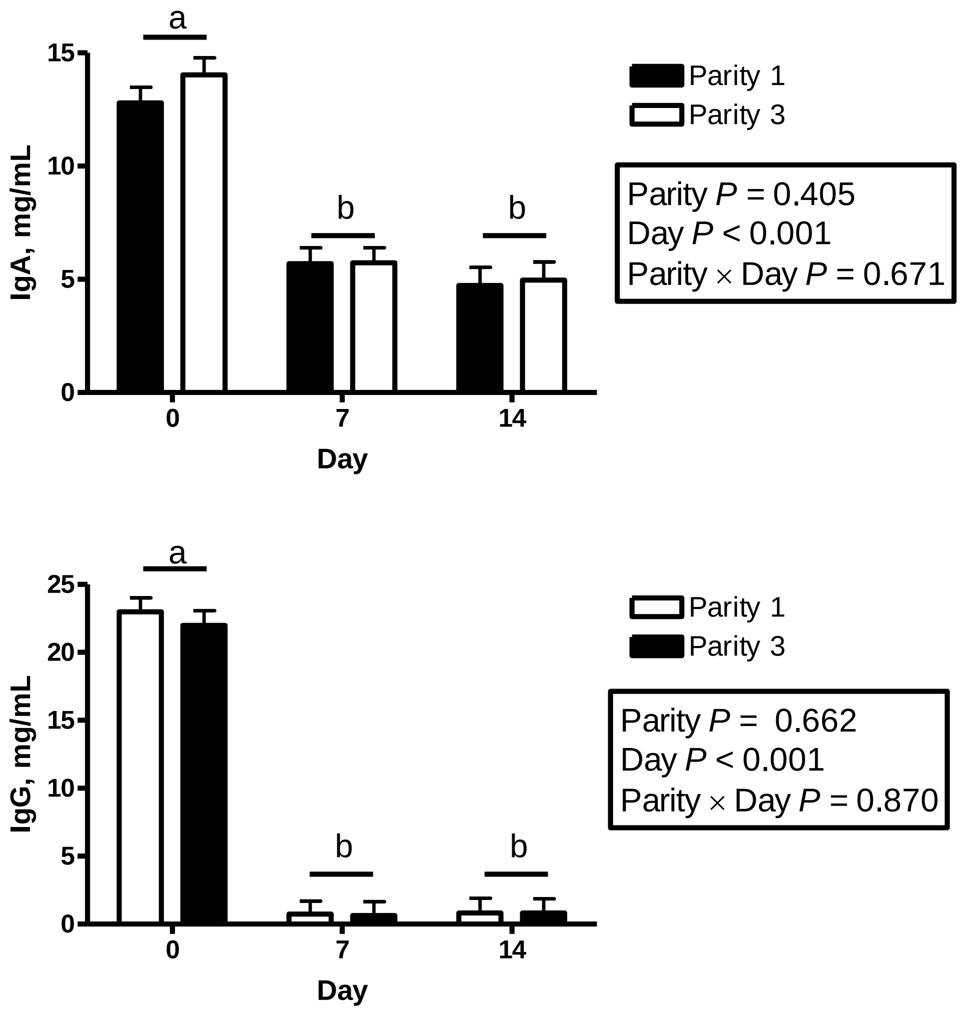
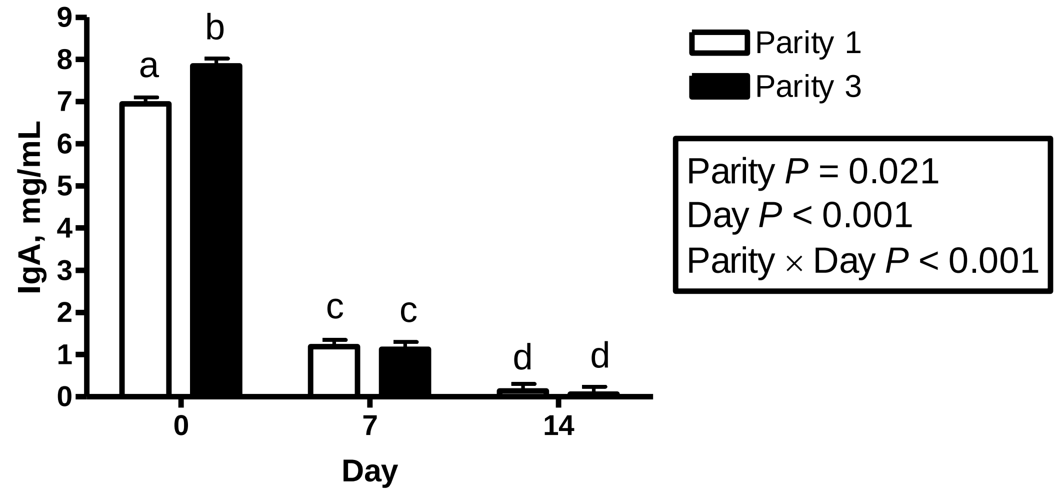


Figure 2. Concentrations of IgA (top panel) and IgG (bottom panel) in colostrum (d 0), and milk samples (d 7 and 14 post-farrowing) obtained from parity (P) 1 and P3 dams. Each bar represents the least-squares mean (± SEM) of 56 and 49 observations for P1 and P3 dams, respectively. Bars without a common letter differ significantly ($P < 0.05$).



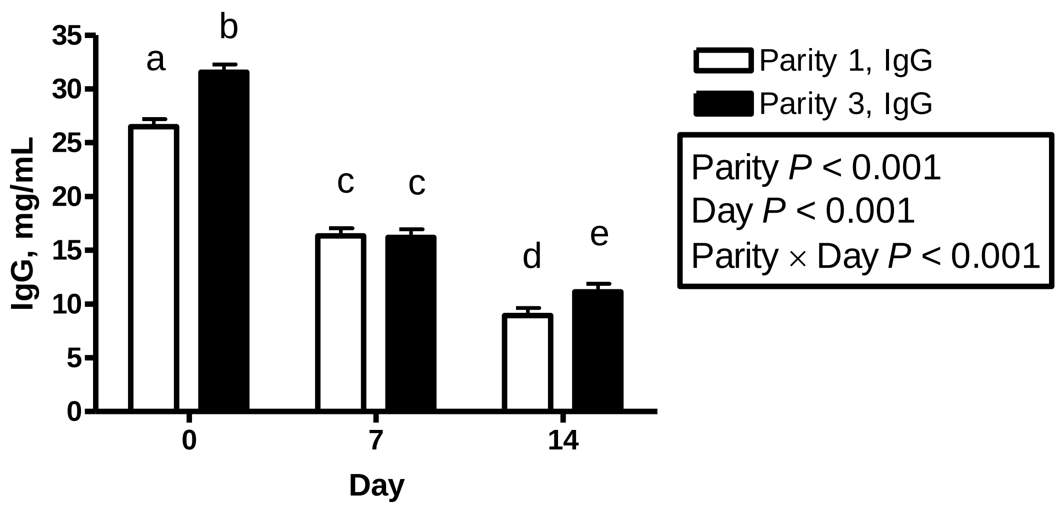


Figure 3. Circulating concentrations of IgA (top panel) and IgG (bottom panel) in serum obtained from the progeny of parity (P) 1 and P3 dams. Immunoglobulin concentrations were evaluated in serum obtained at 0, 7, and 14 d post-farrowing. Each bar represents the least-squares mean (± SEM) of the progeny of 56 and 49 observations for P1 and P3 dams, respectively. Bars without a common letter differ significantly ($P < 0.05$).

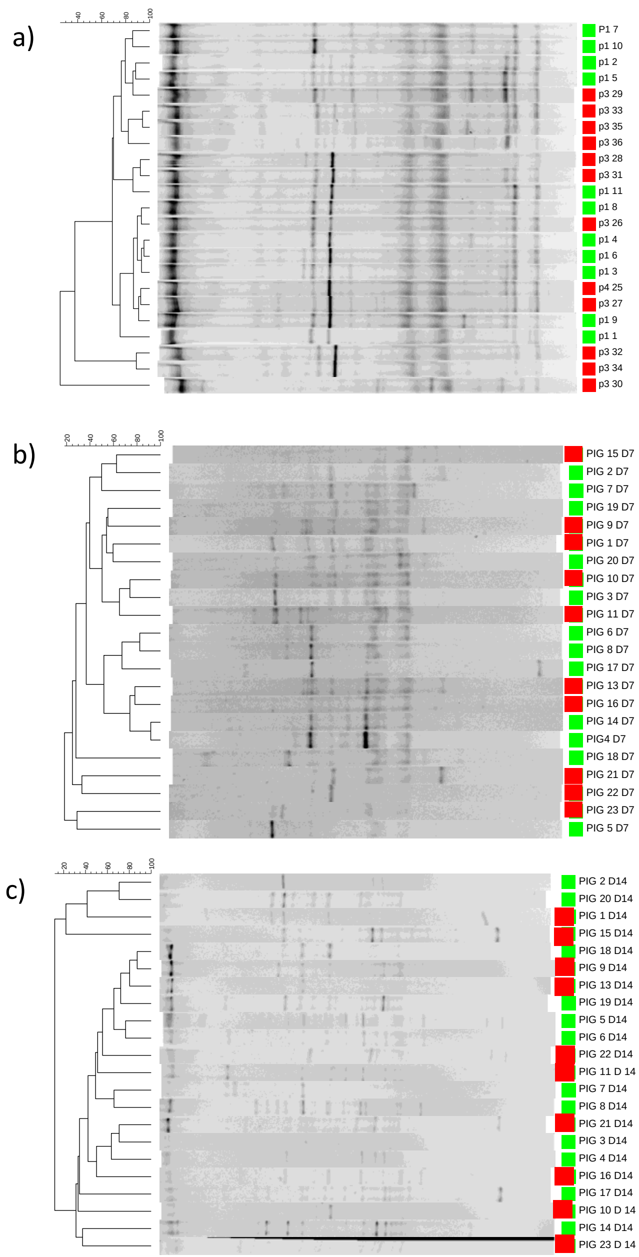


Figure 4. Clustering of microbial profiles of P1 (green square) and P3 (red square) dams (a) and their progeny on d 7 (b) and 14 (c) of lactation. Microbial profiles were analyzed using DGGE. Clustering was performed using UPGMA.

Chapter 4

Effect of dam parity (P1 vs P3) on growth performance and immune parameters of weaned pigs

ABSTRACT: We have previously reported that progeny of parity (P) 3 and P4 dams have increased litter performance and passive immunity compared to P1 progeny during the preweaning period. We therefore, expect progeny of P3 dams to have increased growth performance and immune parameters during the nursery period. The objective of this study was to compare growth performance and immune parameters of P3 and P1 pigs during the nursery phase. Weaned pigs (n=96, initially 5.18 ± 0.05 kg) derived from P1 or P3 dams were allotted to 2 dietary treatments: control (CTL) or antibiotic (AB; 50g/ton Mecadox). Pig BW and feed disappearance were used to determine ADG, ADFI, and G:F. Blood samples were collected on d 0, 7, 14, 21, 28, 35, and 42 for immunoglobulin (Ig) G and A analysis via ELISA. Parity 3 pigs were heavier throughout the nursery period compared to P1 pigs ($P < 0.001$) starting 1 kg heavier (4.6 and 5.7 kg, respectively) and ending 4.7 kg heavier (18.9 and 23.6 kg, respectively). Parity 3 pigs had increased ADG and ADFI during all phases of the nursery period and overall ($P < 0.001$) compared to P1 pigs. Circulating IgA concentrations were increased in P1 pigs compared to P3 pigs ($P = 0.009$). Concentrations of IgA and IgG peaked on d 35, with IgG concentrations lowest on d 7 and 21 and only slightly higher on d 0, 14, 28, and 42. Circulating IgA concentrations were lowest on d 0, then increased as day progressed until peaking at d 35. Dietary treatment affected circulating IgA concentrations as pigs fed the control diet had higher concentrations than pig fed the AB diet. Growth performance, body weights, and immune parameters of nursery pigs are influenced by dam parity and dietary treatment.

Key words: dam parity, growth, immunoglobulins, swine

INTRODUCTION

The transfer of passive immunity is essential for piglet survival during the early stages of life. We have previously shown that the transfer of passive immunity is compromised in piglets from parity (P) 1 dams compared to piglets from P3 and P4 dams (Carney-Hinkle et al., 2012; Carney et al., 2009). We have also shown that these differences in passive immunity may continue after weaning and maybe influenced by adding antibiotics to the diet. Pigs from P4 dams had greater concentrations of IgA when fed antibiotics, while P1 pigs had similar concentrations of IgA when fed the control diet (Carney et al., 2009). Reasons for this interaction are unknown, but passive immunity may play a role.

Differences in passive immunity in the lactation period were greater when comparing progeny of P1 to P3 dams compared to differences observed between progeny of P1 to P4 dams. These greater differences may carry over into the nursery period. We therefore, expect progeny of P3 dams to have increased growth performance and immune status during the nursery period. The objective of this study was to compare the growth performance and immune parameters of P3 pigs and P1 pigs.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska, Lincoln.

Experimental design

A total of 96 weaned pigs (15 d ± .42), derived from P1 or P3 dams (Carney - Hinkle et al., 2012), were used in a 42-d study. To obtain a true representation of BW as the pigs left the farrowing phase, BW was averaged within parity (P1 or P3). Subsequently pigs were selected based on the average BW of each parity to minimize variance. Initial BW of pigs averaged 4.6 and 5.7 kg, respectively for P1 and P3 pigs. Pigs from P1 or P3 dams were chosen from 11 and 7 litters, respectfully.

Six pigs were housed in each pen with 4 pens per treatment with no more than 3 pigs per litter in a pen. Pigs within each parity were sorted by BW and sex (3 barrows and 3 gilts/per pen) within parity then allotted to 1 of 2 dietary treatments, a control diet (CTL) or the CTL diet with antibiotic (AB; 50 g/ton Mecadox). This created a total of 4 dietary treatments (Table 1): 1) P1, CTL; 2) P1, AB; 3) P3, CTL; and 4) P3, AB. All diets were fed in meal form and formulated to meet or exceed NRC requirements for growth (NRC, 1998). Pigs were fed in 3 phases to adjust for nutritional needs: Phase 1 (d 0 to 7); Phase 2 (d 8 to 21); and Phase 3 (d 22 to 42). Pigs were housed in a temperature-controlled room and each pen contained a single nipple waterer and a single self-feeder to facilitate ad libitum access to water and feed. Pig weights and feed disappearance were recorded on d 0, 7, 14, 21, 28, 35, and 42 and ADG, ADFI, and G:F were estimated based on the weekly pen BW and feed disappearance.

Immunoglobulin G and A analysis

Blood samples were collected weekly (d 0, 7, 14, 21, 28, 35, and 42), prior to weighing, from each pig via jugular venipuncture between 0700 and 1000 h. Blood was allowed to clot at room temperature and stored overnight at 4 °C before serum was harvested by centrifugation (20 min at 1,500 × *g*) and frozen (-20˚C) for subsequent analyses. Serum samples for IgG and IgA analyses were diluted (1:100,000 and 1:1,000, respectively for IgG and IgA) prior to analysis. Circulating concentrations of IgG and

IgA in serum were quantified via swine-specific enzyme-linked immunosorbent assays (ELISA; Bethyl Labs Inc., Montogomery TX). The range of the Ig ELISAs was 7.81 to 1,000 ng/mL; sensitivity was 2.0 ng/mL. The intra- and interassay CV for the IgA and IgG ELISAs was 5.81 and 17.78%, and 6.0 and 13.8%, respectfully.

Statistical Analysis

The experiment was a completely randomized design. Pen was considered the experimental unit. All body weight, performance and Ig parameters were analyzed using the MIXED procedure (SAS Inst. Inc., Cary NC), including dietary treatment and parity and their interaction in the model. Body weights were analyzed with and without using d 0 BW as a weighted measure and both analyses are presented. Day was considered a repeated measure for Ig parameters. All means presented are least-squares means.

RESULTS

Pigs were selected as a characteristic sample of how the pigs would normally come out of the nursery. Averaging the pig weights by parity to choose pigs for this study resulted in differences in body weights on d 0 among parities ($P < 0.001$; Table 2) as P3 pigs had heavier BW than P1 pigs. When d 0 weights were weighted, d 0 BW did not affect any body weights after d 0 or growth performance measures. At the end of phase 1, phase 2, and phase 3, P3 pigs were still heavier than P1 pigs ($P < 0.001$; 4.72 and 6.34, 5.54 and 7.73, 7.62 and 10.61 for P1 and P3, respectively). Treatment did not affect pig body weight.

In phase 1, 2, 3, and overall, P3 pigs had increased ($P < 0.002$) ADG and ADFI compared to P1 pigs (Table 2). In phase 1, P3 pigs gained 0.091 kg compared to P1 pigs

0.046 kg (*P* = 0.002). An increased gain of 0.096 kg for P3 pigs was observed in phase 2 (*P*< 0.001) compared to P1 pigs. In phase 3, P3 pigs gained 0.55 kg compared to 0.63 kg in P1 pigs (*P* = 0.001). Overall, P3 pigs had an increased ADG of 0.080 kg more than P1 pigs (*P* < 0.001). In phase 1, (*P* < 0.001) P3 pigs had a 0.077 kg increased feed intake than P1 pigs while in phase 2 (*P* < 0.001) P3 pigs consumed 0.142 kg more feed than P1 pigs. In phase 3, P3 pigs had an ADFI of 0.972 kg compared to P1 0.822 kg ADFI (*P* < 0.001). Overall, ADFI for P3 pigs was 0.136 kg higher for P3 pigs compared to P1 pigs (*P* < 0.001). There was no difference in G:F between parities. Dietary treatment did not affect ADG, ADFI, or G:F.

Circulating Ig concentrations are presented in Figures 1 and 2. Pigs derived from P1 dams had higher concentrations of IgA than P3 pigs (*P* = 0.009). Circulating concentrations of IgA were affected by day (*P* < 0.001; Figure 1a), as concentrations at d 0 were the lowest, but similar to concentrations on d 14. Concentrations of IgA were higher on d 7 than d 0 and 14. Concentrations at d 21 were higher than d 0, 7, and 14, but lower than d 28, 35, and 42. On d 28, IgA concentrations were higher on than d 0, 7, 14, and 21, but lower than d 35 and 42. Concentrations of IgA peaked at d 35 and were slightly lower on d 42. Dietary treatment affected circulating IgA concentrations (*P* = 0.025; Figure 2a) as pigs fed the CTL treatment had higher IgA concentrations than pigs fed the AB treatment.

Immunoglobulin G concentrations in serum were not affected by dam parity (*P* = 0.683; Figure 1b). Circulating concentrations of IgG peaked on d 35 post weaning (*P* < 0.001). Concentrations of IgG on d 0, 7, 14, 21, and 42 were similar. Concentrations of

IgG on d 0, 14, 28, and 42 were also similar. Dietary treatment did not affect circulating IgG concentrations (*P* = 0.663; Figure 2b).

DISCUSSION

The effect of dam parity on nursery pig performance and immune status has not been very thoroughly studied. In this experiment, we found that pigs from P3 dams had increased ADG and ADFI compared to pigs derived from P1 dams. Previously we reported increased ADFI for pigs from P4 dams compared to P1 dams (Carney et al., 2009). As far as the authors know this is the only study that looks at effect of dam parity on nursery pig performance. Pig BW was greater for P3 pigs compared to P1 pigs, which is similar to our previous work that reports greater BW for P4 pigs compared to P1 pigs. Paredes et al. (2012) has reported that parity did not influence pig BW at the end of the nursery period; however, this may be due to limited cross-fostering information across parities.

There was no dietary effect of adding antibiotics to diets on pig performance or BW. Recent studies have found similar results with limited effects of antibiotics on pig performance (Weber et al., 2001; White et al., 2002; Hahn et al., 2006; Walsh et al., 2007; Choi et al., 2011). This may be due to the high health status of the pigs used in these experiments. No differences were found between antibiotic fed and control fed pigs in IgG concentrations, which is very similar to other results (White et al., 2002; Shan et al., 2007; Carney et al., 2009). A significant effect of dietary treatment on circulating IgA concentrations was observed as pigs fed the control diet had higher concentrations than pigs fed the AB diet. Also, P1 pigs had greater concentrations of circulating IgA compared to P3 pigs. In a previous study comparing P4 derived pigs to P1 derived pigs a significant parity by dietary treatment interaction was observed as P1 pigs on the AB diet and P4 pigs on the CTL diet had the lowest circulating IgA concentrations. The reasons for these differences in circulating IgA concentrations could be due to differences in gut microbial communities. Increased circulating IgA concentrations produce increased secretory mucosal IgA concentrations, which control intestinal bacteria communities (Hooper et al., 2012). Dendritic cells in the lamina propria of the gut sample the intestinal bacteria, which interacts with B cells that produce bacteria specific IgA. This specific IgA controls the spread of intestinal bacteria (Cerutti et al., 2011). A change in intestinal bacteria can increase or decrease IgA concentrations. Adding AB to the diet can trigger a change in intestinal bacteria by decreasing bacterial diversity (Jancyk et al., 2007; Kim et al., 2012), therefore possibly changing IgA concentrations.

Growth performance, body weights, and immune parameters in nursery pigs are affected by dam parity. More research is needed to determine if differences in IgA concentrations could be influenced by microbial populations.

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Table 1. Composition of phase 1, 2, and 3 diets (as-fed basis) %.

	Control			Antibiotics[1]		
	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
Ingredient, %	0-7 d	8 - 21 d	22 - 42 d	0-7 d	8 - 21 d	22 - 42 d
Corn	44.35	43.93	60.21	44.35	43.93	60.21
Soybean meal, 47.5% CP	14.75	32.00	33.75	14.75	32.00	33.75
Whey, dried	22.50	15.00	-	22.50	15.00	-
Fish meal	8.00	4.00	-	8.00	4.00	-
Animal plasma	6.00	-	-	6.00	-	-
Corn oil	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	0.40	1.00	1.65	0.40	1.00	1.65
Limestone	0.25	0.35	0.63	0.25	0.35	0.63
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin Premix [2]	0.25	0.25	0.25	0.25	0.25	0.25
Trace Mineral Premix [3]	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine HCl	-	-	0.04	-	-	0.04
DL-Methionine	0.05	0.03	0.03	0.05	0.03	0.03
Mecadox - 2.5 g/lb	-	-	-	1.00	1.00	1.00
Calculated Composition						
CP, %	23.2	23.3	21.2	23.2	23.3	21.2
Total Lys, %	1.56	1.41	1.21	1.56	1.41	1.2
TID[4] Lys, %	1.31	1.26	1.19	1.3	1.25	1.18
Ca, %	0.84	0.79	0.72	0.84	0.79	0.72
P, %	0.81	0.76	0.71	0.8	0.76	0.7
Available P, %	0.59	0.47	0.38	0.59	0.47	0.38
ME, kcal/kg	3488	3448	3453	3454	3414	3418

[1]Antibiotic was supplied as Mecadox 2.5 g/lb, which contained 55 mg/kg of carboadox (International Nutrition, Omaha, NE).

[2] Supplied per kg of diet: vitamin A (as retinyl acetate), 5,500 IU; vitamin D (as cholecalciferol), 550 IU; vitamin E (as α-tocopheryl acetate, 30 IU; vitamin K (as menadione dimethylphyrimidinol bisulfate), 4.4 mg; riboflavin, 11.0 mg; d-pantothenic acid, 22.05 mg; niacin, 33.0 mg; vitamin B_{12} (as cyanocobalamin), 33.0 mg.

[3]Supplied per kg of the diet: copper (as $CuSO_4 \cdot 5H_2O$), 10 mg; iodine (as $Ca(IO_3) \cdot H_2O$), 0.25 mg; iron (as $FeSO_4 \cdot 2H_2O$)), 125 mg; manganese (as MnO), 15 mg; selenium (as Na_2SeO_3), 0.3 mg; and zinc ($ZnSO_4 \cdot H_2O$), 125 mg.

[4] Total ileal digestibility

Table 2. Effect of parity (P) and dietary treatment on average body weight without and with weight on d 0 considered as a weighted variable.

	Dietary Treatments					**P-values**		
	P1[1] Control	**P1[1] AB[2]**	**P3[1] Control**	**P3[1] AB[2]**	**SEM**	**Parity**	**AB[2]**	**Parity × AB[2]**
BW, kg								
d 0	4.93	4.40	5.69	5.72	0.05	< 0.001	0.691	0.773
d 7	4.77	4.67	6.30	6.39	0.08	< 0.001	0.939	0.265
d 21	7.69	7.55	10.60	10.61	0.39	< 0.001	0.788	0.844
d 42	18.93	18.81	23.94	23.27	0.75	< 0.001	0.607	0.723
BW, kg	weighted							
d 0	4.39	4.40	5.69	5.72	0.06	< 0.001	0.716	0.803
d 7	4.77	4.67	6.30	6.39	0.09	< 0.001	0.925	0.270
d 21	7.69	7.55	10.62	10.59	0.30	< 0.001	0.765	0.844
d 42	18.94	18.81	23.95	23.28	0.79	< 0.001	0.596	0.721

[1] P = parity

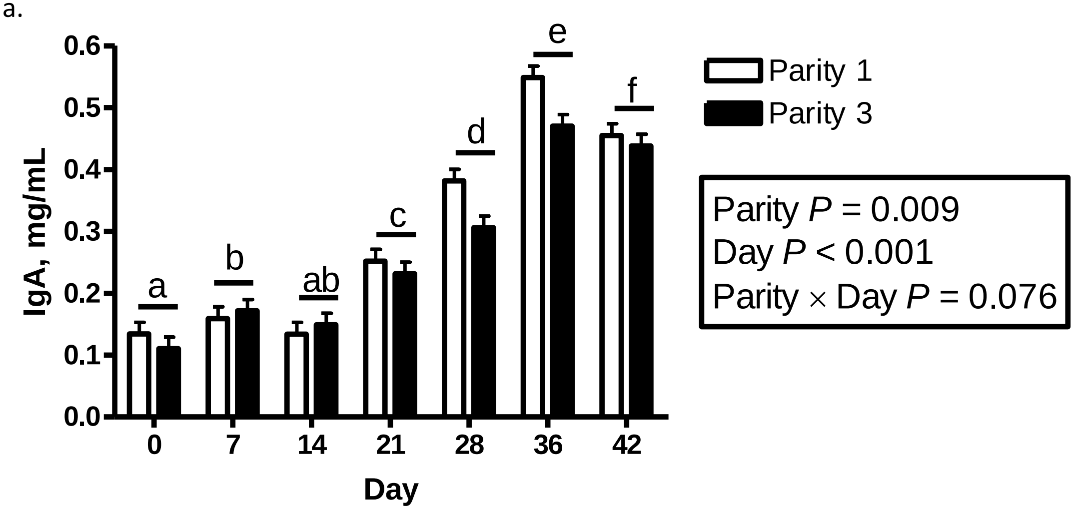
[2] AB = antibiotics (Mecadox 50g/ton)

Table 3. Effect of parity (P) and dietary treatment on ADG, ADFI and G:F of weaned pigs.

	Dietary Treatments					P-values		
	P1[1] CTL[2]	P1[1] AB[3]	P3[1] CTL[2]	P3[1] AB[3]	SEM	Parity	AB	Parity × AB
Phase 1 (d 0 to 7)								
ADG, g	54.17	39.05	87.62	94.88	0.01	0.002	0.733	0.340
ADFI, g	101.1	97.1	179.1	169.0	0.01	< 0.001	0.369	0.911
G:F, g/g	525.6	352.7	487.6	559.1	0.09	0.350	0.570	0.184
Phase 2 (d 8 to 21)								
ADG, g	208.5	208.7	308.5	300.7	0.02	< 0.001	0.822	0.811
ADFI, g	305.7	327.8	468.9	448.1	0.21	< 0.001	0.975	0.335
G:F, g/kg	681.6	631.4	658.4	670.6	0.02	0.636	0.269	0.081
Phase 3 (d 22 to 42)								
ADG, g	535.4	559.6	634.6	619.6	0.02	0.001	0.808	0.311
ADFI, g	798.0	846.0	971.9	971.7	0.25	< 0.001	0.354	0.351
G:F, g/kg	670.6	661.7	653.7	637.4	0.01	0.102	0.300	0.754
Overall (d 0 to 42)								
ADG, g	346.2	351.8	434.8	423.3	0.01	0.003	0.839	0.559
ADFI, g	517.7	542.0	672.1	659.2	0.02	< 0.001	0.7583	0.324
G:F, g/kg	667.9	648.4	647.4	641.9	0.01	0.186	0.219	0.480

[1] P = parity
[2] CTL=pigs fed control diet
[3] AB = pigs fed diet containing antibiotic (Mecadox 50g/ton)

a.



b.

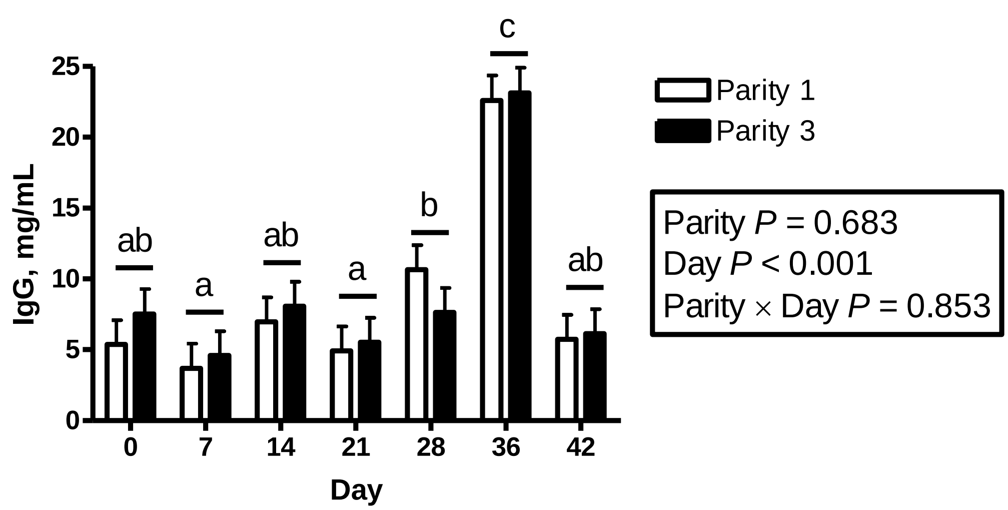
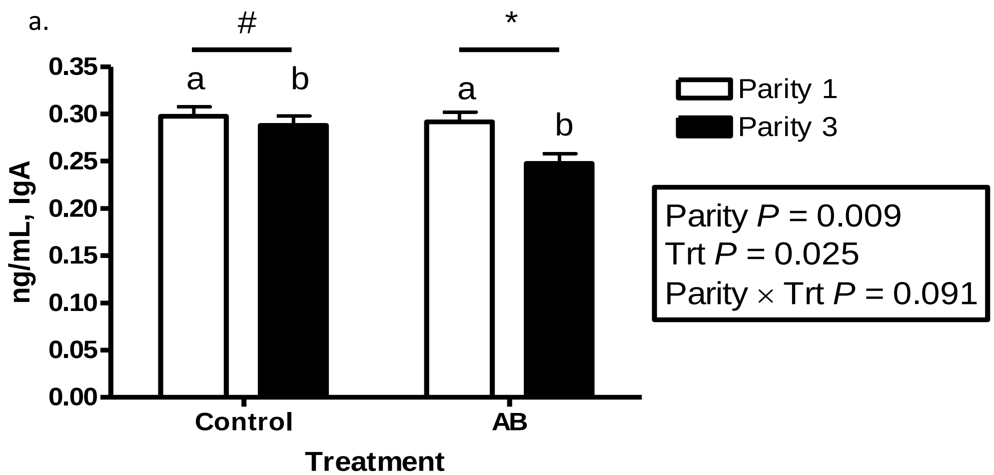


Figure 1 Circulating concentrations of immunoglobulin A (a) and G (b) in serum obtained from the progeny of parity (P) 1 or P3 sows. Immunoglobulin concentrations were evaluated in serum obtained on d 0, 7, 14, 21, 28, 35, and 42 postweaning. Each parity × time bar in panels A represents least square means (± SEM) of serum samples obtained from six pigs per pen at each time point. Bars without a common letter differ significantly ($P < 0.05$).



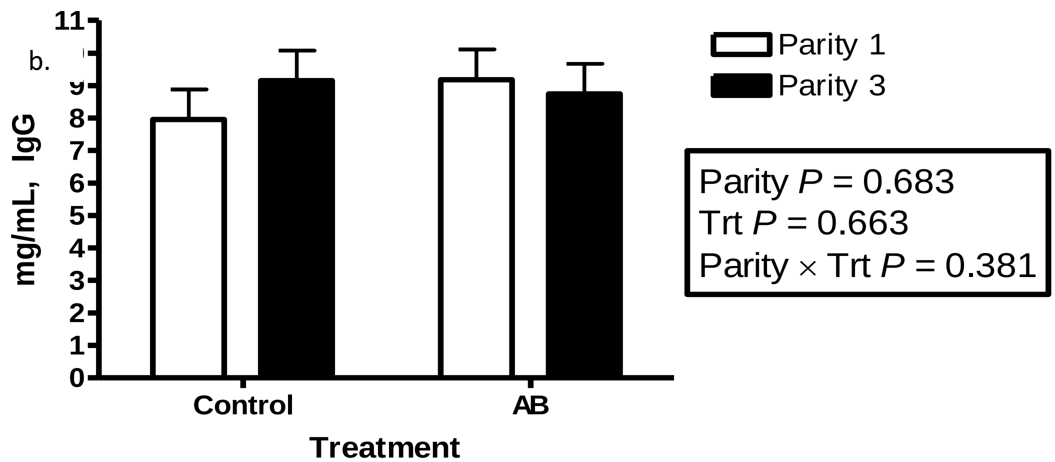


Figure 2. Circulating concentrations of immunoglobulin A (a) and G (b) in serum obtained from the progeny of parity (P) 1 or P3 sows. Progeny were fed a control diet or the control diet with antibiotics (50g/ton Mecadox; AB). Immunoglobulin concentrations were evaluated in serum obtained on d 0, 7, 14, 21, 28, 35, and 42 postweaning. Each parity × trt bar in panels A represents least square means (± SEM) of serum samples obtained from six pigs per pen at each time point. Bars without a common letter or symbol differ significantly ($P < 0.05$).

Chapter 5

Development of swine gut microbiota from birth through the nursery period.

ABSTRACT: Gut microbiota impact host physiology and health. The objective was to evaluate the development of fecal microbiota of pigs from different dam parities (P) from birth through the end of the nursery period (63-d of age). Fecal samples were collected directly from P1 & P3 sows (n = 6/P; d 7 postfarrowing) & 1 pig/litter on d 7, 14, 26 and 61 of age. Pigs were weaned on approximately d 19. Shifts in bacteria were observed by age of pig. Bacteroidetes were decreased in dam and postweaning pigs compared to preweaning pigs, which is inverse of Firmicutes amounts. In dams, Clostridiaceae (33%) were most abundant. Pigs at d 7 had comparable percentages of Bacteroidaceae (14%), Clostridiaceae (11%), Lachnospiraceae (16%), and Lactobacillaceae (19%). Pigs at d 14 had high populations of Bacteroidaceae (13%), and Lachnospiraceae (16%). On d 26 Lactobacillus (31%) was the most dominant followed by Lachnospiraceae (13%). On d 61 Streptococcaceae (25%) were most abundant. Dam parity did not affect gut microbiota. Knowledge of gut microbial establishment may lead to understanding of host-microbial interactions in health and disease.

Keywords: Gut microbiota, swine, early colonization, dam parity

INTRODUCTION

The gastrointestinal tract is colonized by millions of bacteria. The type of bacteria that initially colonize the gut can be influenced by several factors, including type of birth (Dominguez-Bello et al., 2010), environment (Mulder et al., 2009; 2011) and diet. Studies comparing breast-fed versus formula-fed infants and pigs have shown increases in the beneficial bacteria *Bifidobacterium* (Garrido et al., 2012). This is due to the differences in milk composition, mainly the increase human milk oligosaccharides in breast milk (Sela and Mills, 2010).

Gastrointestinal microbiota have been shown to play a role in immune system development. Development of the immune system is dependent upon the ability to differentiate and respond appropriately to commensal and pathogenic microbes that colonize the gastrointestinal tract. Once the immune system has developed, it should be able to differentiate between bacteria which are helpful versus harmful. Thus, the initial bacteria to colonize in the gastrointestinal tract influence the immune systems performance long after its initial development (Hooper et al., 2012).

Our previous work has shown that progeny of parity 3 and 4 sows have increased passive immunity compared to parity 1 progeny. While no differences were observed in milk composition, parity 3 and 4 progeny had much higher concentrations of immunoglobulins A and G than parity 1 progeny, likely due to increased milk yield (Carney et al., 2009a; Hinkle et al., 2011). This difference in transfer of passive immunity may affect gut microbial communities. Comparisons of gut microbial communities between parity 1 and parity 4 progeny using denaturing gradient gel electrophoresis showed increased diversity of parity 1 progeny compared to parity 4 progeny (Carney et al., 2009b). However, when P1 and P3 progeny were compared no differences were observed (Hinkle et al., 2011). A more detailed look at bacterial communities is necessary to determine possible parity differences.

The pig's gut microbial community shifts dramatically upon weaning. While many have looked at the differences in microbial communities at the start of weaning and its initial shift (1 to 2 weeks following weaning) (Leser et al., 2002; Castillo et al., 2006;

Konstantinov et al., 2006; Janczyk et al., 2007; Pieper et al., 2008), few have looked at the colonization of the gut microbial communities from birth to the end of the nursery period (42 d postweaning; d 61of age). We therefore set out to evaluate changes in fecal microbial communities of pigs derived from different dam parities from birth through the nursery period (d 42 postweaning; d 61 of age).

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 (n = 6) and P3 dams (n = 6) which farrowed during a 7-d period beginning September 17, 2009, and ending September 23, 2009. Dams and pigs used in this experiment were of a high health status with no clinical signs of porcine reproductive and respiratory syndrome virus. Dams were co-mingled and housed in stalls during gestation and moved to farrowing crates approximately 5 d prior to their expected farrowing date. On d 0 all piglets were processed including injection of 200 mg Fe (Fe dextran, Uniferon 200, Watchung, NJ), tail-docking, and ear-tagging. No antibiotics were administered to dams or progeny. Boar piglets were castrated before 3 d of age. The entire experiment was conducted at the University of Nebraska Agricultural Research and Developmental Center Swine Unit near Mead, NE. On approximately d 19, pigs were weaned, mixed with other litters, and placed in a nursery facility with a larger cohort of pigs (6 pigs/pen). One pig per pen was then selected for fecal sampling on d 26 and 61 of age. Pig diets were fed in 3 phases:

Phase 1 (d 0 to 7); Phase 2 (d 8 to 21); and Phase 3 (d 22 to 42). Pigs were housed in a temperature-controlled room and each pen contained a single nipple waterer and a single self-feeder to facilitate ad libitum access to water and feed.

Fecal samples were collected from sows on d 7 of lactation. One pig per litter was randomly choosen for fecal collection on d 7 and 14 preweaning. Fecal samples were stored at -20°C until further analysis. Pigs were not creep fed or given antibiotics during the preweaning or postweaning period.

Fecal DNA Extraction.

Extraction of DNA from all fecal samples were carried out according to the methods described by Martinez et al. (2010). Briefly, fecal samples were thawed and a 500 mL aliquot of each sample were used for DNA isolation. All samples were washed with PBS and centrifuged (8,000 × *g* for 5 min at 4°C) 3 times. The bacterial pellet were resuspended in 750 µL Lysis buffer [200 m*M* NaCl, 100 m*M* Tris (pH 8.0), 20 m*M* EDTA, and 20 mg/mL Lysozyme] and transferred to a microcentrifuge tube containing 300 mg of 0.1 mm zirconium. Samples were incubated for 20 min at 37˚C. After incubation, 85 µL of 10% SDS solution and 40 µL Proteinase K (15mg/mL; Sigma) were added to samples followed by another incubation period at 60˚C for 15 min. Phenol:chloroform:isoamyl alcohol (25:24:1, 500 µL) were added and the samples were homogenized in a MiniBeadbeater-8 (BioSpec Products) at maximum speed for 2 min. Layers were separated by centrifugation at 10,000 × *g* for 5 min at 4°C. The top layer were extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1) and twice with chlorophorm:isoamyl alcohol; DNA were recovered by standard ethanol precipitation. Pellets were dried for 30 min at room temperature. The DNA pellet was resuspended in

100 μL of Tris/HCl Buffer (10 m*M,* pH 8.0). The DNA were stored at -20˚C for subsequent analyses.

PCR Amplification

The V1-V3 regions of the 16S rRNA gene were amplified by PCR from fecal DNA. The 16S primers were modified to work with the Roche-454 Titanium adaptor sequences and contain the A or B Titanium sequencing adapter (shown in italics), followed immediately by a unique 8-base barcode sequence (BBBBBBBB) and finally the 5' end of primer. A mixture (4:1) of the primers B-8FM (*5'-CCTCTCCCCTGTGTGCCTTGGAGTCTCGAGTTTGATCMTGGCTCAG-3'*) and B-8FMBifido (*5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGGGTTCGATTCTGGCTCAG-3'*) were used as the forward primer during PCR. As the reverse primer, the primer A-5-18R (*5'-CCTATCTCATCCCTGCGTGTCTCCGACTACTCAGBBBBBBBBATTACCGCGGCTCGTGG-3'*) were used. Individual samples were amplified with primers containing unique barcodes, which will allow mixing of PCR products from multiple samples in a single run, followed by bioinformatics assignation of the sequence to their respective samples via the barcode. The PCR mixture contains 1 µL of reverse primer, 0.25 µL of ex-Taq polymerase (TaKaRa Bio), 1.5 µL of Ex-Taq buffer, 5 µL of deoxynucleotides and 37 µL of sterile dH_20 were used for the reaction. The PCR program consists of an initial denaturing step for 5 min at 95°C, followed by 30 cycles of denaturation at 95°C for 45 sec, annealing at 57°C for 45 sec and extension at 72°C for 10 min. The PCR products quantified based on their staining intensity using the image acquisition software

Genesnap (Syngene USA). PCR products were combined in equal amounts and gel purified using the QIAquick Gel Extraction Kit (Qiagen).

Pyrosequencing was performed by the Core for Applied Genomics and Ecology (CAGE, University of Nebraska) from the A end with the 454/Roche A sequencing primer kit using a Roche Genome Sequencer GS-FLX following manufacturer's protocol for the Rosche 454 GS FLX Titanium. The sequences were cleaned and checked for chimeras using Qiime software (Caporaso et al., 2010) before submission to sequence analysis (below). Sequences were binned according to barcode using the Ribosomal Database Project (RDP) Pyrosequencing Pipeline (http://pyro.cme.msu.edu/) with default parameters (which include the removal of sequences containing at least one ambiguous nucleotide), except for the minimum sequence length, which was set to 300 bp.

Sequence analysis to characterize microbial populations

Two independent approaches were used to analyze the sequences obtained with pyrosequencing. First, the Classifier tool (with a minimum bootstrap value of 80%) of the RDP was applied to obtain a taxonomic assignment of all sequences. This approach allows a fast determination of the proportions of bacterial groups at different taxonomic levels (phylum, family, genus). Second, sequences were assigned Operational Taxonimic Units (OTU) that are quantified in individual subjects. OTU were aligned and classified using UCLUST in Qiime software.   OTU that contain less than 3 sequences were excluded from the analysis. OTU were subjected to taxonomic classification and grouped according to phylum using Qiime (Firmicutes, Bacteroides, and Actinobacteria). Within these phyla, random sequences of each OTU identified above were aligned with the most closely related type strains (97% or higher similarity) in the NCBI database using Muscle

3.6. Phylogenetic trees were built with MEGA 5.0 software by neighbor-joining with 1,000 bootstrap replicates. These trees allow us to visually assign OTU as sequence clusters which, in most cases, encompass sequences from several subjects, and consensus sequences were generated for each OTU.

Microbial community composition of the fecal samples and the digesta were determined using 16S rRNA tag sequencing. The sequence data generated were analyzed using two different approaches which includes OTU based analysis and phylotype based analysis. In OTU based analysis, the sequences were aligned and clustered at 97% similarity using Qiime, and the alpha diversity of the microbial communities were determined using Shannon's and Chao1 indexes, which estimate species richness and species diversity respectively. Beta diversity of the microbial communities was analyzed using unifrac, principal coordinate analysis, and non-metric multi-dimentional scaling. In addition, rarefaction curves were generated to estimate sampling depth and adequacy. In phylotype based analysis, the reads were classified using "classifier" to identify taxonomic assignments and were analyzed to identify phylotype difference among and between samples.

Statistical Analysis

All data were analyzed as a randomized complete design using dam as the experimental unit. Analysis of variance was performed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model for alpha diversity and taxonomy included day, parity, and their interaction. Least squares means were calculated for each independent variable.

RESULTS

Phylogeny

Pyrosequencing of 16S rRNA amplicons from 60 samples resulted in an average of 8,448 sequences per sample after quality control (506,904 sequences in total) with mean sequence length of approximately 495 bp. The average number of OTU indentified per subject was 6,928.

The majority of sequences were assigned to four phylum, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. The percentage of each phylum differed by age of pig. Firmicutes were the most abundant phylum at all ages. Sows and post-wean pigs had the highest percentages (87.4%, 87.0%, and 87.3 %, for sows, d 26 and 61 pigs, respectfully) while prewean pigs had lower percentages (71.2 % and 62.3% for d 7 and 14 prewean pigs, respectfully). The opposite was observed for the phylum Bacteroidetes. The largest percentage were observed in prewean pigs (16.9 and 26.4% for d 7 and 14 pigs, respectfully) and smaller amounts in sows and postwean pigs (4.4, 3.6%, and 7.8%, for sows, d 26 and 61 pigs, respectfully). The amount of Proteobacteria changed as pigs grew, with the largest percentages occurring during lactation (2.2 and 6.6% for d 7 and 14, respectfully) and much smaller amounts occurring after weaning (1.3 and 0.6%, for d 26 and 61, respectfully). Sows had a small amount of Proteobacteria (1.0%). Actinobacteria also fluctuated with age. Pigs on d 7 of lactation had the greatest amount (4.5%), which then dipped considerable during on d 14 of lactation (0.4%). A small jump occurred on d 26 of age (3.0%) but decreased again by the end of the nursery period (d 42, 0.7%). Sows had very little Actinobacteria (0.6%).

Firmicutes

The amount of Firmicutes varied by age of the pig, with the largest variation occurring at the lowest taxonomic levels. At the family level, the taxa that occurred most often included Lactobacilliaceae, Streptococcaceae, Clostridiaceae, Lachnospiraceae, Ruminococcaceae, and Erysipelotriaceae (Table 1). Sows were dominated by Clostridiaceae (33.7%), followed by smaller amounts of Lactobacillaceae (10.8%), Ruminococcaceae (7.7%), Lachnospiraceae (3.8%), Erysipelotrichaceae (3.0%) and Clostridiales cluster XII (1.8%). Clostridiaceae was almost completely composed of *Clostridium* (26.6%). When OTU were identified, several were related to *Clostridium* species, including *Clostridium roseum* (7.1%), *Clostridium glycolicium* (5.5%), and *Clostridium baratii* (2.4%; Table 2). Lactobacillaceae was almost completely composed of *Lactobacillus* (10.8%), with most of the genus being composed of bacteria closely related to *Lactobacillus amylovorus* (8.8%; Table 2). Bacterial species were spread out within Ruminococcaceae, Lachnospiraceae, Erysipelotriaceae, and Cluster XII, as no dominate species were identified.

Bacteria classifications were similar through the lactation period (d 7 and 14 preweaning), with Lactobacillaceae, Lachnospiraceae, Clostridiaceae, Streptococcaceae, Ruminococcaceae, and Erysipelotichaeceae found in both days, though with varying amounts. On d 7 preweaning, the greatest amount of bacteria were classified as Lactobacilliaceae (18.7%) and Lachnospiraceae (16.5%), followed by Clostridiaceae (10.1%), Streptococcaceae (6.4%), Ruminococcaceae (5.6%), and Erysipelotrichaceae (1.3%). On d 14 preweaning, the greatest amount of bacteria was Lachnospiraceae (16.2%), followed by Clostridiaceae (9.1%), Erysipelotrichaceae (7.6%), Lactobacillaceae (6.3%), Ruminoccoaceae (5.6%), and Streptococcaceae (2.9%). Also appearing at this time is *Enterococcus* (2.2%). Lactobacilliaceae is again dominated by *Lactobacillus* (18.6 and 6.2%, for d 7 and 14 preweaning, respectfully). At the OTU level occurred between days as bacteria closely related to *L. amylavorus* amount was high on d 7 (5.6%), but very little if any found on d 14 (0.2%). Also found during the lactation period were species related to *L. delbrueckkii* (4.6 and 0.8%, d 7 and 14, respectfully; Table 2). Clostridiaceae was dominated by *Clostridium* (6.9 and 6.2 %, d 7 and 14, respectfully), with bacteria related to *C. bolteae* (3.2 and 2.3 %, d 7 and 14, respectfully), and *C. scindens* (2.8 and 2.0%, d 7 and 14, respectfully) being the most prevalent. *Streptococcus* (6.4 and 2.9%, d 7 and 14, respectfully) was the dominant genus in Streptococcaceae, with bacteria related to *S. pasteurianus* (3.2 and 2.3%, d 7 and 14, respectfully) observed. No dominant genus was found within the families Lachnospiraceae, Ruminococcaceae, and Erysipelotrichaeceae during lactation.

Weaning causes a very large shift in bacterial communities due to diet changes. On d 26 of age, Lactobacillaceae was the most dominate family (31.4%), followed by Lachnospiraceae (13.4%), Ruminococaceae (13.2%), Clostridiales cluster XIV and XII (2.9 and 8.4%, respectfully), Enterococcaceae (2.7%), and Erysipelotrichaceae (1.29%). *Lactobacillus* (31.2%) was the prevalent genus with *L. amylovorus* (20.0%) being the most dominant species. No significant genus were found within the families Ruminococaceae, Clostridiaceae, Enterococcaceae, and Erysipelotrichaceae. Within the Lachnospiraceae family *Blautia* was highly prevalent (8.3%).

On d 61 of age, the end of the nursery period, the diet of the pig had changed twice, as more animal protein ingredients were removed and replaced with plant proteins.

This change made the family Streptococcaceae the dominant family (28.8%). Present in smaller amounts was Lactobacilllaceae (9.8%), Lachnospiraceae (8.8%), Ruminococcceae (8.6%), Clostridium cluster XIV (6.1%), Clostrideaceae (5.6%), Veillonellaceae (4.9%), and Erysipelotrichaceae (3.5%. Streptococcaceae was predominantly composed of bacteria in the *Streptococcus* genus (24.8%), which was primarily composed of *S. alactolyticus* (19.5%). *Lactobacillus* (9.8) genus occurred at a high percent, with most of this amount composed of *L. amylovorus* (6.7%). *Blautia* (6.1%) was again found at a high amount of Lachnospiraceae.

Bacteroidetes

The percentages of Bacteroidetes also varied by age, as much higher percentages occurred during the lactation period, compared to sows and postwean pigs. At the family level, Bacteroidaceae was dominant during the lactation period, with a majority of bacteria belonging to the *Bacteroides* genus (14.0 and 13.2%, d 7 and 14, respectfully; Table 1). When OTU were compared, bacteria related to *Bacteroides dorei* occurred at a high percent (6.7%) on d 7 preweaning, but was almost absent (0.57%) on d 14 preweaning. No other significant *Bacteroides* OTU were identified. On d 61 *Prevotella* was occurred in greater amounts than any other day (5.8%).

Actinobacteria

Actinobacteria was present in 96% (58/60) samples, though in very small amounts. The largest percentages were observed in d 7 preweaning and postweaning pigs. In d 7 preweaning pigs, *Actinomyces* (3.2%) was most prevalent Actinobacteria.

Postweaning pigs on d 26 were spread out between Bifidobacteriaceae (1.6%) and Coriobacteriaceae (1.4%; Table 1).

Proteobacteria

Proteobacteria were present in 90% (54/60) of pigs, with the smallest percent observed in d 61 postweaning pigs. The most prominent bacteria in this phylum are classified in the genus *Esherichia/Shigella* (Table 1). The highest percentage of *Esherichia/Shigella* was observed on d 14 preweaning (5.6%).

Diversity

Rarefaction curves were generated by plotting the number of phylotypes against the number of newly identified sequences. The rarefaction curves did not reach a plateau at the genus and species levels, indicating that the number of OTU was likely to increase with additional sampling (Figure 2).

Diversity was also measured by looking at species richness, using Chao and Shannon's indexes) as well as species evenness (Simpson's Index). Age and parity did not affect species richness or evenness (Table 3).

Dam Parity does not affect gut microbial communities

The effect of dam parity on gut microbiota was evaluated. Dam parity (parity1 vs parity 3) did not affect gut microbial diversity or taxonomy.

DISCUSSION

This study explored the development of the gut microbiota of pigs from birth to the end of the nursery period (d 61 of age). At birth pigs are born sterile, but colonization occurs very rapidly. We explored how the bacteria that colonize the gut change as the pig develops, starting in the lactation period (d 7 and 14), through the weaning period (d 26) and finishing at the end of the nursery period (d 61). From birth to the end of the lactation period pigs primarily consume sows milk, which nutritional content changes as lactation progresses. At weaning pigs were transported to a separate nursery facility and fed a diet high in plant and animal sources. The diets were then adjusted to meet the pig's nutritional requirements at d 26 and 42 postweaning, so a total of 3 diets were fed (Phase I, II, and III). These changes in environment as well as dietary changes could influence how the gut microbiota are established.

The pig gut is primarily composed of Firmicutes. The amount and type of Firmicutes, however, changed with the age of the pig. The amount of Firmicutes was lowest during the lactation period, but was inversely related to the increase in Bacteroidetes and Actinobacteria. This is likely due to the diet of the pig, milk. Human infants have increased numbers of Bacteroidetes and Actinobacteria compared to human adults [16]. This increase has been linked to these bacteria's ability to metabolize milk oligosaccharides (MO; Sela and Mills, 2010). While human MO are much more complex, porcine MO have been found to be more similar to human MO than bovine MO (Tao et al., 2010). The amount of bacteria in pigs during lactation has previously been published (Poroyko et al., 2010). This study reports greater amounts of Bacteroidetes (49%) than we observed (16.8 and 26% on d 7 and 14, respectfully) and decreased Firmicute amounts (42% versus 71 and 62%, for d 7 and 14, respectfully). Poroyko et al. (2010)

observed the predominant genus within Bacteroidetes being *Prevotella* and *Oscillibacter,* while the dominant Firmicutes were *Clostridium* and *Lactobacillus*. We, however, found the dominant Bacteroidete to be *Bacteroides* with very small amounts of *Prevotella* and found no *Oscillibacter*. The genera found within Firmicutes were similar as we also found the dominant genus to be *Clostridium* and *Lactobacillus.* The time points and sampling methods of this study versus our study is different. We observed pigs fecal microbiota at d 7 and 14 of age, while Poryoko et al. (2010) observed pig cecal microbiota n d 21. These differences may account for the discrepancies. It is also possible that Bacteroidetes continues to increase throughout lactation, as we saw increasing amounts with age and Poroyoko et al. (2010) saw an even greater amount at an older age, but more research is needed to determine if this is true. In addition, differences in environment and the diet of the sow, as sow diet can change milk composition, could also account for differences between these two studies.

After weaning the amount of Bacteroidetes decrease and Actinobacteria and Firmicutes increase. The increase in Actinobacteria is short, as it is only observed on d 26 and very little is observed on d 61. This increase is due to the increased amount of *Bifidobacterium*. This was also observed by Franklin et al. (2002). *Bifidobacterium* growth has been associated with the gut microbiota of human infants consuming breast-milk. This is likely again due to the MO content of the milk (Sela and Mills, 2010). After weaning, to ease the transition of weaning, pig diets contain a large amount of whey. Whey is added to the diet to increase the concentration of lactose in the diet, and to, again, ease the transition to a solid diet for the pig. Interestingly, the breakdown of lactose by lactase in commercial settings can result in an intermediate called galactooligsaccharide (GOS; Lamsal, 2012), which has been shown to stimulate *Bifidobacterium* growth (Smiricky-Tjardes et al., 2003; Tzortizis et al., 2005; Davis et al., 2011; Walton et al., 2012). However, *Bifidobacterium* has been shown to grow in the presence of lactose as well (Rockova et al., 2011). This could account for the increase in *Bifidobacterium* observed on d 26 of age, seven days after weaning.

The changes in *Lactobacillus* following weaning has previously been studied. The changes in amount of *Lactobacillus* varies from study to study. Some studies report decreases (Franklin et al., 2002; Su et al., 2008), no change (Pieper et al., 2008), or increases (Janczyk et al., 2010) as we observed. Previous studies have also identified *Lactobacillus* species changes after weaning. Similar to what we observed, *L. amylovorus* became the dominant species after weaning (Janczyk et al., 2007; Pieper et al., 2008). Decreases in *L. salivarius* and *L. acidophilus* after weaning occurred, while *L. reuteri* was variable with high amounts on d 5 post weaning and decreased again on d 11 (Janczyk et al., 2007; Pieper et al., 2008). The later species, *L. salivarius, L. acidophilus, L. reuteri* were not found to be dominant species in our pig feces on d 26 of age.

There was another shift in bacteria from d 26 to d 61. The most significant shift that occurred is the increase in *Streptococcus*, particularly *S. alactolyticus*. This species has previously been observed as the most abundant OTU in pigs of a similar age (Leser et al., 2002). Other studies that observed pigs at a similar age also reported Firmicutes as the most abundant phyla, followed by Bacteroiedetes, Proteobacteria, and Actinobacteria (Mulder et al., 2009; Schmidt et al., 2011), however, these studies report much higher amounts of Proteobacteria (18% vs. 0.6%) and lesser amounts of Firmicutes (70% vs.

87%). Similar families and genus were observed at the family level between studies, including increased *Prevotella* (Mulder et al., 2009; Schmidt et al., 2011).

By d 61 of age the gastrointestinal tract and immune system should be reaching full maturity. Therefore, we expected the gut microbiota of these pigs to be similar to the sow gut microbiota. In fact, no differences between sows and pigs at d 61 were observed at the phylum level. At the family level five families (Clostridium cluster XIV, Lachnospiraceae, Prevotellaceae, Streptococcaceae, and Veillonellaceae) were higher on d 61 pigs compared to sows, however, sows had increased amounts of Clostrideaceae. Due to the experimental design it is impossible to determine why these differences occurred, but it is possibly due to the change in environment of pigs, as pigs were moved to a separate facility from the sows at weaning. However, it could also be due to differences in diet or age of the pig.

Surprisingly, dam parity had no effect on the gut microbiota of the pig at any age. We have previously reported that dam parity increased the transfer of passive immunity in older parity (3 or 4) dams (Carney-Hinkle et al., 2012; Manuscript 1 & 2). Progeny of parity 3 dams had increased serum immunoglobulin (Ig) G and A levels. The increase in Ig levels, especially IgA, as its main site of action is the gut, could affect gut microbial populations as the immune system and gut microbiota influence on another (Hooper et al., 2012). That does not seem to be the case however, as no difference in dam parity were observed.

In conclusion, the development of the pig gut microbiota was observed. Pigs during lactation had similar bacteria to human infants, which is likely due to the milk diet. After weaning, as expected, the gut microbiota shifts with the changes in diet. However, we were unable to determine when pigs reach a mature gut microbiota due to the experimental design, so more research is needed for this to be determined. Dam parity did not affect gut microbiota despite differences in immune status of the pigs. Knowledge of gut microbial establishment in pigs may lead to a better understanding of host-microbial interactions in health and disease.

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Table 1.Phylogeny of gut microbiota (%) in swine from sows and progeny from birth to d 61 of age (end of nursery period).

			Preweaning		Postweaning	
Phylum	Bacterial taxa	Sows	d 7	d 14	d 26	d 61
Actinobacteria		0.36	2.90	0.55	1.31	0.54
	Actinomycetaceae	0.01	3.24	0.10	0.00	0.00
	Actinomyces	0.01	3.23	0.10	0.00	0.00
	Bifidobacteriaceae	0.15	0.82	0.20	1.59	0.39
	Coriobacteriaceae	0.49	0.32	0.12	1.35	0.34
Bacteroidetes		5.63	10.96	25.40	2.37	4.72
	Bacteroidaceae	1.56	13.96	13.21	0.03	0.00
	Bacteroides	1.56	13.96	13.21	0.03	0.00
	Porphyromonadaceae	0.45	0.48	1.26	1.46	0.41
	Prevotellaceae	1.48	1.63	1.81	0.67	6.76
	Prevotella	0.62	0.60	0.68	0.35	5.80
Firmicute		83.77	81.48	61.66	93.01	91.48
	Lactobacillaceae	10.85	18.73	6.30	31.40	9.76
	Lactobacillus	10.82	18.60	6.22	31.20	9.69
	Enterococcaceae	0.05	0.09	2.19	2.73	0.02
	Enterococcus	0.05	0.07	2.02	2.63	0.00
	Streptococcaceae	0.12	6.35	2.92	0.27	24.84
	Streptococcus	0.11	6.34	2.92	0.27	24.77
	Clostridiaceae	33.73	10.08	9.11	0.97	5.59
	Clostridium	26.65	6.93	6.17	0.44	3.21
	Incertae Sedis XIII	1.84	0.28	0.54	2.92	0.34
	Incertae Sedis XIV	0.14	0.85	0.17	8.36	6.06
	Lachnospiraceae	3.81	16.46	16.24	13.37	8.75
	Blautia	0.10	0.77	0.11	8.35	6.05
	Peptostreptococcaceae	0.04	0.07	0.01	0.06	0.05
	Ruminococcaceae	7.68	5.61	5.63	13.20	8.56
	Erysipelotrichaceae	2.97	1.30	7.63	1.29	3.46
	Veillonellaceae	0.34	0.40	0.43	0.87	4.87
Proteobacteria		0.82	0.76	5.77	0.11	0.09
	Enterobacteriaceae	0.93	1.95	5.66	1.20	0.21
	Escherichia/Shigella	0.89	1.90	5.55	1.17	0.19

Table 2. Dominant phylogenetically blasted operational taxonomic units (OTU) in gut microbiota of swine from sows and progeny from birth to d 61 of age (end of nursery period).

		Preweaning		Postweaning	
Species/OTU	Sows	7	14	26	61
Bacteroides dorei	0.06	6.70	0.57	0.00	0.00
Clostridium baratii	4.91	0.01	0.03	0.00	0.00
Clostridium bolteae	0.02	3.02	4.16	0.00	0.00
Clostridium glycolicum	5.14	0.01	0.01	0.03	0.77
Clostridium roseum	7.21	0.00	0.02	0.23	2.42
Clostridium scindens	0.00	2.82	2.01	0.06	0.00
Lactobacillus amylovorus	8.83	5.64	0.18	20.00	6.69
Lactobacillus delbrueckii	0.09	4.59	0.81	0.09	0.18
Shigella flexneri	0.70	1.36	3.70	0.79	0.11
Streptococcus alactolyticus	0.00	0.00	0.00	0.01	19.51
Streptococcus pasteurianus	0.03	3.20	2.31	0.10	0.00

Table 3. Diversity indexes for species richness (Chao and Shannon’s) and evenness (Simpson’s) by age and parity.

		Diversity Index		
		Chao[1]	Shannon's[2]	Simpson's[3]
Parity 1	Sow	6010	7.89	0.97
	d 7	4660	7.07	0.94
	d 14	5687	6.73	0.91
	d 26	6546	7.46	0.95
	d 61	6414	6.73	0.93
Parity 3	Sow	5975	7.27	0.90
	d 7	6406	7.47	0.93
	d 14	6893	7.68	0.95
	d 26	6564	7.14	0.90
	d 61	5681	7.69	0.95
	SEM	810	0.45	0.02
	Parity	0.39	0.34	0.37
	Day	0.78	0.92	0.96
	Parity × day	0.55	0.29	0.21

[1]Chao – similar to Shannon’s index, but uses unequal probabilites to estimate species richness

[2]Shannon’s - measures the probability that any two organisms will be the same phylotype by taking into account number of species and richness of species

[3]Simpson’s - measures the species evenness by taking into account the number of species

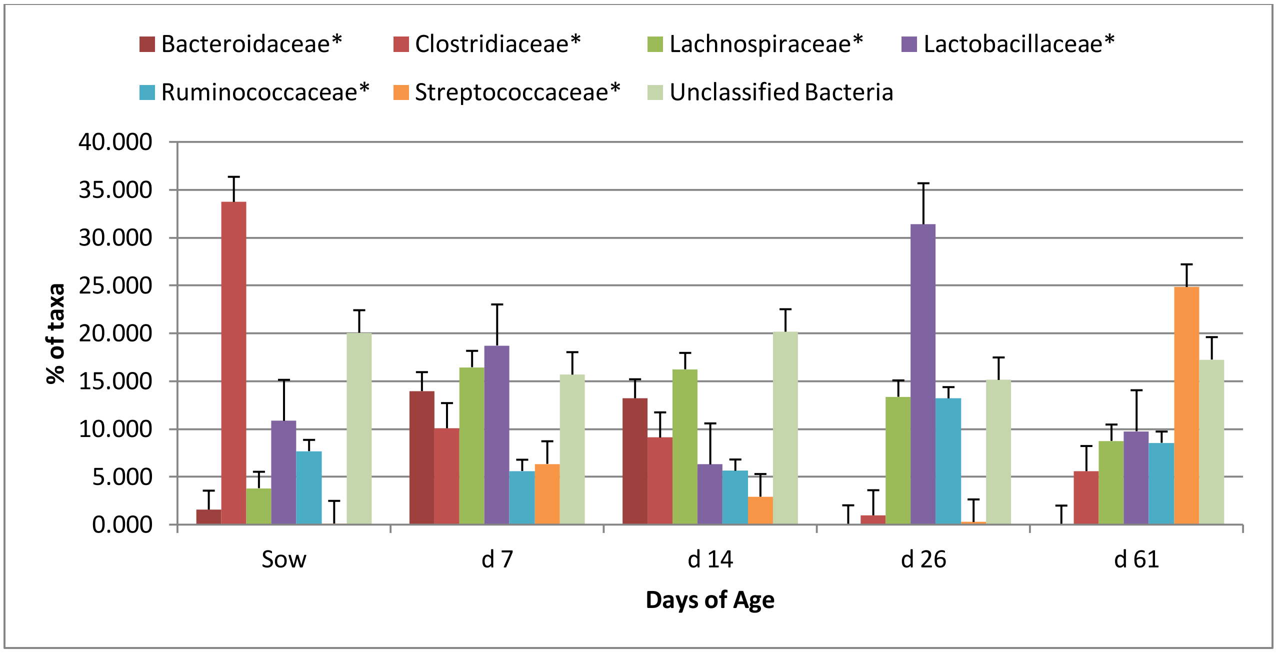

Figure 1. Fecal bacteria populations of sows, and progeny on d 7, 14, 26 and 61 of age, taxonomically classified at the Family level. Significant changes by day are noted with a (*) following the Family classification.

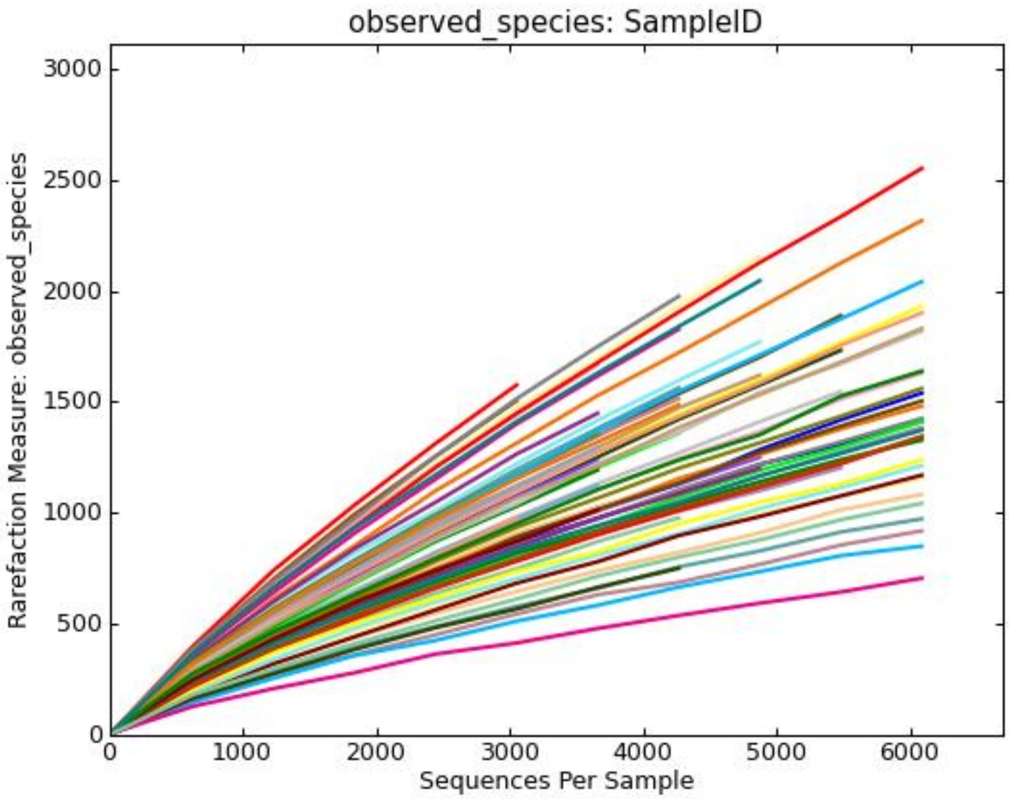

Figure 2. Alpha rarefaction curves of OTU in individual samples. OTU were identified using 97% cutoffs for rarefaction. Rarefaction curves look at diversity of individual samples. A higher rarefaction curve on the Y axix represents more diversity. In order to determine if a fair representation of all possible OTU were identified, is is ideal for curves to plateau. None of these curves plateau.

Chapter 6

Effects of fostering across dam parity on progeny growth performance and passive immunity

ABSTRACT: Previous research indicates that progeny growth and health may be affected by dam parity. The objective was to determine if differences in dam parity are due to in utero growth and development or due to colostrum and milk intake by comparing parity 1 and parity 3 dams and their progeny. Dams (n=18) were assigned to 1 of 4 treatments: 1) Parity 1 dam with P1 progeny (P1P1), 2) Parity 1 dam with P3 progeny (P1P3), 3) Parity 3 dam with P1 progeny (P3P1), 4) Parity 3 dam with P3 progeny (P3P3). Litter performance data was collected and all progeny were weighed on d 0, 7, and 14 preweaning, weaning and d 7 and 14 postweaning. Colostrum and milk samples were collected from dams on d 0, 7, and 14 of lactation and immunoglobulin (Ig) A and G concentrations were quantified. Decreased mortality was observed for P3P3 piglets compared to piglets fostered across parities (P1P3 and P3P1; $P = 0.019$). On d 0, P3P3 piglets had greater BW compared to all other treatments ($P = 0.005$). At d 14 postweaning, P1P1 piglets had decreased ($P = 0.08$) BW compared to all other treatments. Decreased ($P > 0.05$) concentrations of IgA were observed in P1P3 dams on d 0 of lactation compared to all other dams, which corresponded with the decreased ($P = 0.008$) circulating IgA concentrations in their progeny. Compared to all other treatments on d 0 and 7 preweaning, P3P3 piglets had greater ($P = 0.008$) serum IgA concentrations. In addition, P1 piglets fostered on to either P1 or P3 dams (i.e., P1P1 and P3P1 piglets) had lower ($P < 0.001$) serum IgA compared to P3 piglets fostered on to either P1 or P3 dams on d 7. During the lactation period, pigs had similar ADG despite compromised immunity of P1 raised progeny. Therefore, in utero growth and development had a larger impact on growth rate than passive immunity.

Keywords: dam parity, *in utero* growth, passive immunity

INTRODUCTION

We have previously shown that progeny from P4 dams have increased performance compared to progeny from P1 dams. More pronounced differences in performance were observed between P3 and P1 progeny. Differences in growth performance and health among progeny derived from different dam parities may be attributed to multiple factors (e.g., host genetics, immunity, and others). To account for these differences, we analyzed immunoglobulin (Ig) concentrations in dam colostrum and milk and progeny serum. Immunoglobulins are the most important immune components in colostrum and milk as they provide passive immunity before gut closure as well as continued immune protection throughout lactation (Rooke and Bland, 2002). We found no differences in concentrations of IgG and IgA in sow colostrum and milk when we compared P1 to P4 dams (Carney et al., 2009a) or P1 to P3 dams (Hinkle et al., 2011a). Despite no differences in colostrum and milk there are differences in progeny serum concentrations. For example, P4 progeny had greater serum IgG compared to P1 progeny (Carney et al., 2009a). This was also observed when comparing P3 progeny to P1 progeny (Hinkle et al., 2011). We observed no differences in serum IgA concentrations in progeny derived from P4 or P1 dams (Carney et al., 2009a); however, when P3 progeny were compared to P1 progeny, P3 progeny had significantly greater serum IgA concentrations (Hinkle et al., 2011). From this data it was concluded that progeny from mature dams (i.e., greater than P2) benefit from greater passive transfer of immunity compared to progeny from P1 dams.

Despite the differences observed between dam parity in passive immunity, it is still unknown if the differences observed between dam parity progeny growth performance are due to initial body weights or the transfer of passive immunity. Therefore, for this experiment the objective was to foster progeny across dam parity to determine if the differences observed in dam parity are due to *in utero* growth and development or due to colostrum and milk intake by comparing P1 and P3 dams and their progeny.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska, Lincoln.

Animals, Experimental Design, and Dietary Treatments

Eighteen (n = 9 P1 and 9 P3) sows were brought to the farrowing facilities in Lincoln, NE at approximately d 107 of gestation. All sows had ad libitum access to a common lactation diet. Sows were assigned to 1 of 4 treatments: 1) Parity 1 sow with P1 progeny (P1P1); 2) Parity 1 sow with P3 progeny (P1P3); 3) Parity 3 sow with P1 progeny (P3P1); and 4) Parity 3 sow with P3 progeny (P3P3). All piglets were fostered to a different dam to remove any effect of fostering. Piglets were removed from their dam and moved to the foster dam before suckling colostrum. It was important for sows to begin farrowing in close intervals. In order to achieve this, all sows were induced on d 112 of gestation with Lutalyse. After the initial fostering, pigs were not fostered again to standardize litters. Litter performance data was collected including live born, stillborn, mummified fetuses, total born, initial genetic litter body weight, number weaned, fostered on litter body weight, litter weight at d 7 or weaning, litter gain from d 0 to 7, 8 to 14, and 14 to weaning (averaged d 19), and mortality. All progeny were weighed on d 0, 7, 14, and at weaning (averaged d 19) to determine body weight, litter weight, litter gain, and ADG. At weaning all piglets were moved to the nursery and assigned pens by litter without mixing pigs. All pigs were fed a common diet and treatments were set as listed above. Pigs and feeders were weighed on d 0, 7, and 14 post weaning to determine ADG, ADFI, and G:F. At d 14 postweaning pigs were diagnosed with an *Escherichia Coli* infection and the trial was terminated.

Colostrum and milk samples were collected on d 0, 7, and 14 of lactation from all sows from all functional teats. Samples were stored at -20°C until further analysis. Serum samples were collected from all piglets from each litter via jugular venipuncture on d 0, 7, 14, and weaning, and d 7 and 14 post weaning. All samples were stored at -20°C until further analysis.

Milk and serum analyses

Colostrum and milk samples were diluted (1:50,000) and concentrations of IgA and IgG were quantified as described below. Piglet serum samples were diluted for IgG and IgA analyses (1:100,000 and 1:1,000, respectively). Concentrations of IgA and IgG in serum, colostrum, and milk were quantified via swine-specific enzyme-linked immunosorbent assays (ELISA; Bethyl Labs Inc.). The intra- and interassay CV for the IgA and IgG ELISAs was 3.93 and 11.01%, and 5.94 and 8.61% respectfully for serum and milk combined.

Statistics

The experiment conducted as a completely randomized design. Treatment structure was considered a 2 ×2 factorial for dam parity and litter parity. Dam was considered the experimental unit with the model including effects of dam parity, litter parity, and day and their interactions. All data were analyzed using the MIXED procedure of SAS. Day was considered a repeated measure. A random statement was included when analyzing individual pig BW and progeny Ig concentrations to ensure litter was the experimental unit. Litter sizes were found to be different between treatments, so litter size was used as a weighted measure on litter performance including number weaned, litter body weight at all time points, litter gain, and mortality.

RESULTS

Litter performance results are presented in Table 1 and 2. No differences in live born, stillborn, mummified fetuses, total born, or initial genetic litter body weight were found between P1 and P3 sows. After cross fostering occurred there was a tendency for the P3 litters to have smaller litters, irrespective of nurse dam (P1 or P3). Therefore, fostered on litter size was used as a weighted measure to account for different litter sizes for all remaining effects. No differences were observed in number weaned or fostered on litter body weight. For piglet mortality, significant dam parity by litter parity interaction was observed, as P3P3 piglets had decreased mortality compared to piglets fostered across dam parity (P1P3 and P3P1). Wean age was affected by treatment ($P < 0.001$), as P1P1 pigs were weaned about 4 days earlier than pigs from all other treatments.

Individual piglet body weights are presented in Table 3. At birth P3P3 piglets had greater BW ($P = 0.01$) compared to progeny that were fostered across dam parity (P1P3 and P3P1). At weaning (average d 19) progeny of P3 dams were heavier than P1 progeny (*P* = 0.04) irrespective of nurse dam. In addition, at weaning progeny fostered to P3 dams (i.e., P3P1 and P3P3) had greater (*P* = 0.04) BW compared to piglets fostered to P1 (i.e., P1P3 and P1P1). On d 7 postweaning, piglets fostered to P3 dams had greater (*P* = 0.009) BW compared to progeny fostered to P1 dams. On d 14 postweaning, P1P3 and P3P3 had greater BW (*P* = 0.02) compared to P1P1 and P3P1 piglets. At approximately d 14 postweaning, piglets became infected with *E. Coli*, so the remainder of the experiment was terminated. Wean age likely played a role on the differences seen in dam parity postweaning, as P1P1 pigs had a 4 d earlier wean age compared to all other treatments. Therefore, we are not able to determine if dam parity actually played a role in pig BW after d 14 preweaning, as BW is confounded with wean age.

Piglets fostered on P3 dams (P3P1 and P3P3) had greater ADG (*P* = 0.01) from d 15 to weaning compared to piglets fostered on P1 dams (P1P3 and P1P1). From weaning to d 7 postweaning P1P3 piglets were observed to have a negative ADG (-1.56 g of gain) resulting in a sow parity by litter parity interaction. Piglets fostered on to nurse dams of the same parity (i.e., P1P1 and P3P3 piglets) had greater ADG (*P* = 0.01) compared to progeny fostered to nurse dams of the opposite parity (i.e., P1P3 and P3P1).

Concentrations of IgA and IgG in colostrum and milk are presented in Figure 1. A significant dam parity x litter parity x day interaction was observed for IgA concentrations as P1P3 dams had lower (*P* = 0.02) concentrations of IgA on d 0 of lactation compared to all other treatments. The concentrations of IgA for P1P3 on d 0 were similar to all other concentrations across all treatments on d 7 and 14 of lactation.

Colostrum and Milk IgG concentrations were not affected by parity, but as expected IgG concentrations were greatest on d 0 with decreased concentrations on d 7 and 14.

Concentrations of piglet circulating Ig are presented in Figure 2. Progeny circulating IgA concentrations follow the same trend as the colostrum and milk concentrations, as a dam parity x litter parity x day interaction was observed. Parity 3 piglets fostered on to P1 dams (P1P3) had the lowest IgA concentrations, while P3P3 had the greatest concentrations at d 0 (P = 0.008) among all treatments. On d 7 preweaning, P3P3 piglets had greater IgA serum concentration than piglets fostered to P1 dams (P1P1 and P1P3), while P3P1 piglets had intermediate IgA concentrations. Piglet serum IgG concentrations were not affected by dam parity; however, a significant day effect was observed, as expected. Specifically, piglet IgG concentrations were observed to be at their peak on d 0 and decreased throughout the experiment (P < 0.001).

DISCUSSION

This experiment was conducted to determine if effects of dam parity were due to differences in dam parity or *in utero* growth performance. Previous research done by others and at our facilities have reported increased litter performance and piglet gain in more mature dams compared to first parity dams (Hendrix et al., 1978; Wilson and Johnson, 1980; Mahan, 1991, 1994, 1998, 2000; Kemme et al., 1997; Averette et al., 1999; Peters and Mahan, 2010; Quesnal et al., 2008; Smits et al., 2011; Carney-Hinkle et al., 2012). It was however, unclear whether these differences were due to the immaturity and size of the dam or the passive immunity provided by the dam. To determine this we conducted a fostering experiment where all progeny were fostered to either a P1 or a P3 dam.

In order for progeny to be fostered at a reasonable time it was essential that dams were synchronized on d 112 for parturition to start at the same time. Unfortunately, not all dams reached d 112 of gestation at the same time. This then created problems with standardizing litter size and wean age, as all dams had to be weaned at the same time. After standardizing litters P3 litters tended to be smaller. Also, P1P1 progeny were weaned at 16.6 ± 0.6 d, while all other treatments were weaned around 20.3 ± 0.07 d, causing performance differences due to wean age. Wean age has a large effect on pig performance through the nursery and finisher phases. It has previously been observed that increasing weaning age, increases ADG and decreases mortality during the nursery phase, as well as increases ADG in the finishing phase (Main et al., 2004; Davis et al., 2006). The early wean age also compromised immunity as early weaned pigs had decrease white blood cells and cellular immunity (Blecha et al., 1983; Davis et al., 2006).

Using litter size as a weighted measure corrected *P*-values for litter performance, however, differences in body weight due to wean age were not able to be corrected. This was determined by comparing ADG of pigs between treatments. No parity × day interaction was observed for P1P1 pigs which indicated pigs were growing at the same rate as all other P1 pigs or pigs on P1 dams. The P1P1 pigs as the smallest throughout the nursery period because of the 4 d difference in wean age between P1P1 and all other treatments. Therefore, any growth differences observed after d 14 preweaning are confounded with weaning age. All information following d 14 preweaning was then ignored to draw conclusions.

At birth P3P3 pigs were bigger than all other treatments. Throughout lactation, the P3P3 pigs also had increased circulating IgA concentrations compared to all other treatments. These pigs therefore had an advantage over all other pigs. When then turn to our other treatments to determine if dam size or passive immunity played a larger role in pig performance.

No differences in birth weight were observed between P1P1, P1P3, and P3P1 pigs. Parity 1 dams with P3 progeny, however, had unusually low colostrum IgA concentrations, which compromised their passive immunity and decreased circulating IgA concentrations. Despite the compromised immunity, no differences in body weight or ADG were observed between these three treatments. It is therefore, likely that during the lactation period, birth weight (*in utero* growth) played a larger factor in growth performance than passive immunity.

Due to circumstances surrounding this experiment (wean age and litter size) the interpretation of this data was difficult. Two conclusions can be drawn, however. First, progeny fostered to P1 dams had lower circulating IgA concentrations than progeny fostered onto P3 dams. Second, despite the severely compromised immunity in P1P3 pigs, they still gained at a similar rate as the P1P1 and P3P1 treatments. We can then make an overall conclusion that initial body weight (*in utero* growth) plays a larger factor on growth rate during the lactation period.

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Table 1. Dam parity effect on initial litter performance before fostering

No. of Sows	7	8	*P* - Values	
Parity	1	3	SEM	Parity
Born Live	12.38	10.63	1.445	0.393
Stillborn	1.21	2.125	0.663	0.332
Mummified Fetuses	1.00	0.25	0.355	0.149
Total Born	14.58	12.50	1.714	0.392
Litter Birth Weight[1]	13.06	14.01	2.406	0.775

[1] Calculated birth weight before pigs were fostered.

Table 2. Dam parity effect on litter performance after fostering.[1]

						P - Value		
Dam Parity	1	1	3	3				Dam ×
Litter Parity	1	3	1	3	SEM	Dam Parity	Litter Parity	Litter Parity
No. of Sows	3	4	4	4				
Litter size	12.92	10.80	11.58	9.00	1.41	0.232	0.085	0.857
Mortality	0.90[ab]	2.40[a]	1.93[a]	0.00[b]	0.65	0.297	0.735	0.019
No. pigs weaned	10.02	6.40	7.64	7.00	1.44	0.500	0.122	0.267
Wean age	16.63[a]	20.61[b]	20.39[b]	20.00[b]	0.07	0.044	0.025	0.004
Piglet BW, kg								
Birth	1.26[a]	1.18[a]	1.09[a]	1.48[b]	0.07	0.378	0.040	0.005
d 7 pre-wean	2.41[ab]	2.16[a]	2.00[a]	2.73[b]	0.19	0.633	0.186	0.016
d 14 pre-wean	4.14[a]	3.83[a]	3.73[a]	4.96[b]	0.27	0.181	0.098	0.012
Weaning	4.75	5.77	5.75	7.10	0.40	0.010	0.009	0.670
d 7 post-wean	5.15	6.00	5.73	7.68	0.36	0.009	0.002	0.148
d 14 post-wean	5.40	5.69	6.24	8.01	0.42	0.002	0.025	0.088
ADG, g								
d 0 to d 7 pre-wean	161.1[ab]	137.4[ab]	124.8[a]	179.0[b]	20.11	0.887	0.428	0.059
d 8 to d14 pre-wean	242.0[ab]	230.5[a]	238.5[a]	311.3[b]	14.53	0.019	0.052	0.012
d 15 to weaning	225.6	271.8	297.9	355.5	24.75	0.007	0.048	0.812
Wean to d 7 post-wean	61.8[ab]	43.8[ab]	14.0[a]	88.4[b]	17.38	0.925	0.124	0.020
d 8 to d14 post-wean	20.6	9.5	76.3	42.8	30.79	0.156	0.460	0.708

[1] Means without a common letter within a row differ significantly ($P < 0.05$).

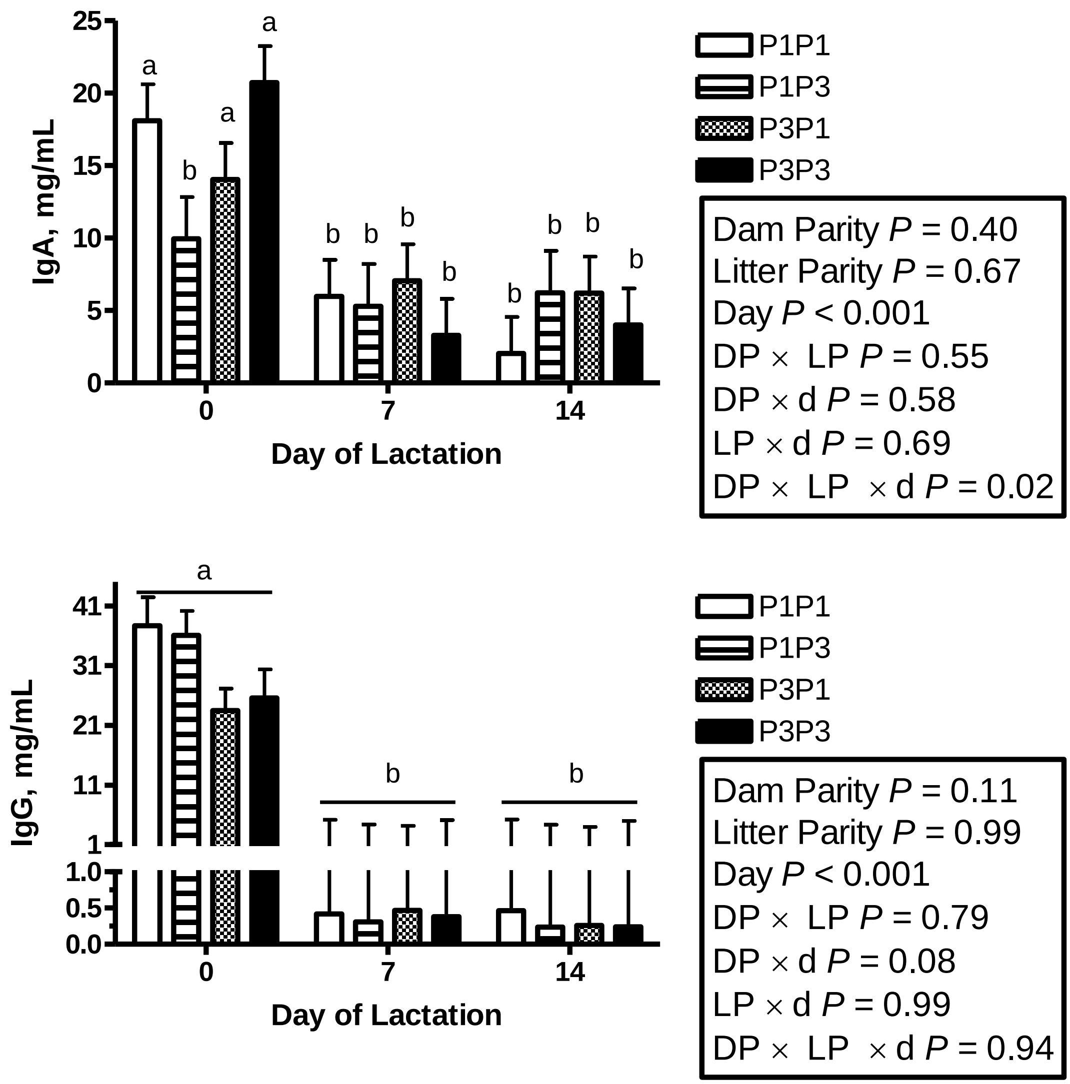
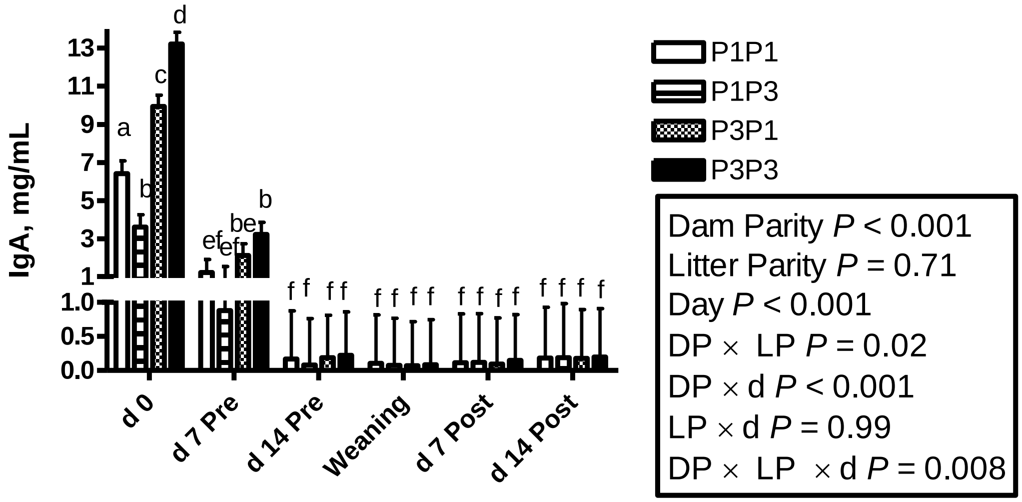


Figure 1. Colostrum and milk Immunoglobulin (IgA and IgG) concentrations in progeny of P1 and P3 dams fostered to P1 or P3 dams. Treatments are 1) Parity 1 dam with P1 progeny (P1P1), 2) Parity 1 dam with P3 progeny (P1P3), 3) Parity 3 dam with P1 progeny (P3P1), 4) Parity 3 dam with P3 progeny (P3P3). Means without a common letter differ significantly ($P < 0.05$).



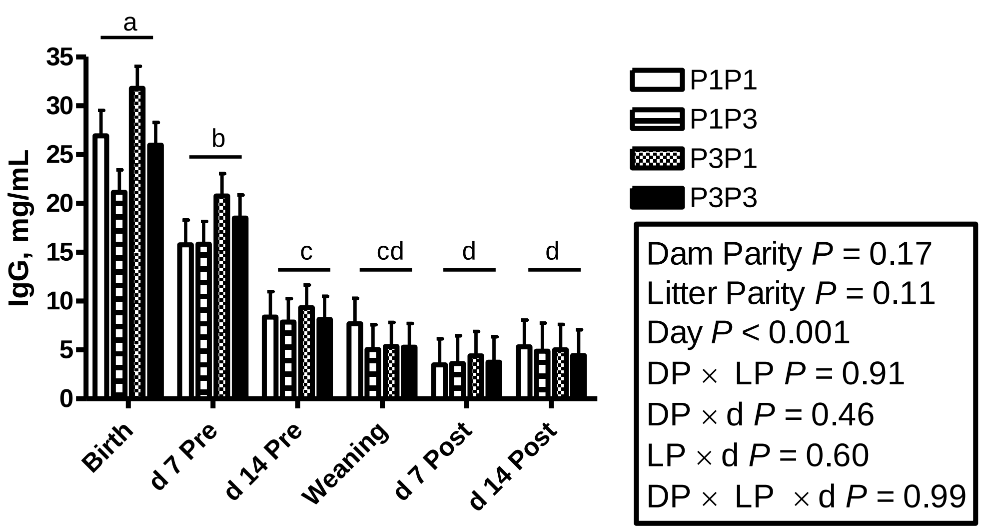


Figure 2. Circulating Immunoglobulin (IgA and IgG) concentrations in progeny of P1 and P3 dams fostered to P1 or P3 dams. Treatments are 1) Parity 1 dam with P1 progeny (P1P1), 2) Parity 1 dam with P3 progeny (P1P3), 3) Parity 3 dam with P1 progeny (P3P1), 4) Parity 3 dam with P3 progeny (P3P3). Means without a common letter differ significantly ($P < 0.05$).