BOARD INVITED REVIEW: The pig microbiota and the potential for harnessing the power of the microbiome to improve growth and health[1]

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ABSTRACT: A variety of microorganisms inhabit the gastrointestinal tract of animals including bacteria, archaea, fungi, protozoa, and viruses. Pioneers in gut microbiology have stressed the critical importance of diet:microbe interactions and how these interactions may contribute to health status. As scientists have overcome the limitations of culture-based microbiology, the importance of these interactions has become more clear even to the extent that the gut microbiota has emerged as an important immunologic and metabolic organ. Recent advances in metagenomics and metabolomics have helped scientists to demonstrate that interactions among the diet, the gut microbiota, and the host to have profound effects on animal health and disease. However, although scientists have now accumulated a great deal of data with respect to what organisms comprise the gastrointestinal landscape, there is a need to look more closely at causative effects of the microbiome. The objective of this review is intended to provide: 1) a review of what is currently known with respect to the dynamics of microbial colonization of the porcine gastrointestinal tract; 2) a review of the impact of nutrient:microbe effects on growth and health; 3) examples of the therapeutic potential of prebiotics, probiotics, and synbiotics; and 4) a discussion about what the future holds with respect to microbiome research opportunities and challenges. Taken together, by considering what is currently known in the four aforementioned areas, our overarching goal is to set the stage for narrowing the path towards discovering how the porcine gut microbiota (individually and collectively) may affect specific host phenotypes.

Key Words: gastrointestinal, health, microbiome, nutrient–microbiome interactions, pigs

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INTRODUCTION

The gastrointestinal tract (**GIT**) is involved in several physiological processes including, but not limited to, nutrient digestion, absorption, and metabolism and 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 the microbiota has the potential to lead to numerous chronic diseases and disorders. Therefore,

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knowledge of the interactions of the GIT and the microbiota with nutrient utilization is essential for advancement in feeding our livestock species to increase growth performance and health status. In addition, these advancements also have potential for increasing the knowledge base relative to potential applications in human disease.

The microbiome contains a highly diverse population that may vary from individual to individual, but provides functional redundancy through similar gene profiles (Huttenhower et al., 2012). Individuals may have niche microbes and functions that allow for special and unique microbial arrangements that can be masked due to broad classifications of microbes and genes (Lozupone et al., 2012). Our understanding of the composition and role that the microbiome plays has increased in the last few decades with advances in genome sequencing and the "-omics" (e.g., metabolomics, proteomics, and transcriptomics). As we have gained knowledge with respect to gut microbial composition, we have also learned that many factors (e.g., age, diet, environment, and genotype) affect microbial composition and this in turn has profound effects on animal health and disease.

The intent of this review is to discuss what is currently known with respect to microbial colonization of the porcine GIT, the impact of nutrient (protein, lipids, and carbohydrates) by microbe interactions on growth and health, the therapeutic potential of prebiotic, probiotic, and synbiotic applications, and finally, to discuss the challenges and opportunities in future microbiome research.

MICROBIAL COLONIZATION OF THE PORCINE GASTROINTESTINAL TRACT

As an example, we have included a table (Table 1) indicating the dynamics of bacterial taxa (phylum, family, and genus level) colonization over time (sows and pigs, days 7 to 61 of age) from fecal samples collected from pigs in 2 different experiments (Hinkle et al., 2012; Tran et al., 2018). Colonization of the neonatal piglet gut commences immediately following birth (Fouhse et al., 2016). Colonization of the neonatal GIT by maternal and environmental microbes is instrumental in physiological and immunological development of the gut. These initial colonizers are mostly aerobic or facultatively anaerobic bacteria such as *Escherichia coli*, *Shigella flexnerii*, and *Streptococcus* spp. (Konstantinov et al., 2006; Fouhse et al., 2016). These organisms consume available oxygen, thus creating an anaerobic environment conducive to the growth of anaerobes such as *Bacteroides*, *Clostridium*, *Bifidobacterium*, and *Lactobacillus* spp. (Konstantinov et al., 2006; Petri et al., 2010; Fouhse et al., 2016).

Several longitudinal studies have investigated the temporal patterns associated with the establishment of the gut microbiota in pigs. Using a cloning approach for 16S rRNA gene libraries, Petri et al. (2010) studied the microbial succession patterns in digesta collected from the stomach, small intestine, and the colon of neonatal pigs. Members of the families Clostridiaceae and Enterobacteriaceae were the major colonizers early on (up to about 0.5 d post-parturition) but were subsequently displaced by Streptococcaceae members. Streptococcaceae remained the dominant family between 1 and 3 d before they were displaced by members of the family Lactobacillaceae, which remained the dominant bacterial group for the remainder of the study (20 d of age). Interestingly, the authors observed that the bacterial succession pattern was similar across all the gastrointestinal locations sampled in the study (i.e., stomach, small intestine, and colon; Petri et al., 2010).

Hinkle et al. (2012) studied the development of the gut microbiota of pigs from birth to the end of the nursery period using fecal samples and high-throughput amplicon-based DNA sequencing. The sampling scheme covered the lactation period (days 7 and 14 of age), weaning period (days 26 of age), and the end of the nursery period (days 61 of age). Changes in abundance were observed in the phylum Firmicutes with the age of the pigs, although Firmicutes remained the predominant phylum at all ages. Previous research had also shown that Firmicutes, along with Bacteroidetes, were the 2 major bacterial phyla of the porcine gut regardless of age of the animals (Mach et al., 2015). In the work by Hinkle et al. (2012), the abundance of Firmicutes was lowest during the lactation period (71.2% and 62.3% on days 7 and 14, respectively) and abundance increased following weaning (87.0% and 87.3% on days 26 and 61, respectively). An opposite trend was observed for members of the phylum Bacteroidetes, where the highest abundances were observed for preweaned piglets (16.9% and 26.4% on days 7 and 14, respectively), whereas postweaning piglets harbored lower relative abundances of these bacteria (3.6% and 7.8% on days 26 and 61, respectively). At the genus level, *Bacteroides* was dominant during the lactation period (days 7 and 14) while there was an increase in the genus *Prevotella* at the end of the nursery period (day 61). This postweaning increase in *Prevotella*

Table 1. Example of the dynamics of bacterial taxa (phylum, family, and genus level) colonization over time (sows and pigs days 7–61 of age) from fecal samples collected from pigs in 2 different experiments

	Relative abundance (%)									
	Exp. 1	Exp. 1	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 2	Exp. 2	Exp. 2	Exp. 1
Taxon	Sows	d 7	d 14	d 21	d 26	d 28	d 35	d 42	d 49	d 61
Phylum: Firmicutes	83.77	81.48	61.66	59.49	93.01	79.57	82.30	90.32	93.02	91.48
Family Lactobacillaceae	10.85	18.73	6.30	1.50	31.40	20.43	39.05	17.15	4.87	9.76
Genus: *Lactobacillus*	10.82	18.60	6.22	1.50	31.20	20.43	39.04	17.14	4.86	9.69
Family: Streptococcaceae	0.12	6.35	2.92	0.54	0.27	1.08	0.21	41.16	52.04	24.84
Genus: *Streptococcus*	0.11	6.34	2.92	0.54	0.27	1.07	0.21	41.11	51.98	24.77
Family: Clostridiaceae	33.73	10.08	9.11	3.88	0.97	1.27	5.36	4.48	10.74	5.59
Family: Lachnospiraceae	3.81	16.46	16.24	10.20	13.37	33.74	18.22	9.35	9.81	8.75
Family: Ruminococcaceae	7.68	5.61	5.63	4.75	13.20	7.63	6.75	4.86	4.09	8.56
Family: Erysipelotrichaceae	2.97	1.30	7.63	17.54	1.29	1.35	0.45	0.22	0.21	3.46
Family: Veillonellaceae	0.34	0.40	0.43	1.52	0.87	0.45	2.06	1.65	0.12	4.87
Phylum: Bacteroidetes	5.63	10.96	25.40	27.93	2.37	5.03	5.34	5.20	1.63	4.72
Family: Bacteroidaceae	1.56	13.96	13.21	16.01	0.03	0.00	0.00	0.00	0.00	0.00
Genus: B*acteroides*	1.56	13.96	13.21	15.65	0.03	0.00	0.00	0.00	0.00	0.00
Family: Prevotellaceae	1.48	1.63	1.81	2.05	0.67	0.90	1.86	1.49	1.01	6.76
Phylum: Proteobacteria	0.82	0.76	5.77	7.42	0.11	0.03	0.03	0.00	0.00	0.09
Family: Enteroacteriaceae	0.93	1.95	5.66	6.96	1.20	0.03	0.02	0.00	0.00	0.21
Genus: *Escherichia*	0.89	1.90	5.55	6.76	1.17	0.03	0.02	0.00	0.00	0.19
Phylum: Actinbacteria	0.36	2.90	0.55	0.05	1.31	3.10	3.38	0.09	0.08	0.54
Family: Bifidobacteriaceae	0.15	0.82	0.20	0.01	1.59	3.10	3.36	0.09	0.08	0.39
Genus: *Bifidobacterium*				0.01		3.10	3.36	0.09	0.08	

Exp. 1. Hinkle et al., 2012.

Exp. 2. Tran et al., 2018.

abundance is in agreement with previous research (Mach et al., 2015). The increase in *Prevotella* may have been in response to the pig diet containing a higher amount of plant-derived components at this stage, as previous research has shown high abundances of *Prevotella* in humans who consume a mainly plant-based diet (De Filippo et al., 2010; Simpson and Campbell, 2015). In addition, Hinkle et al. (2012) observed a large shift in the bacterial community due to the dietary changes associated with the change from a liquid diet (i.e., sow's milk) to solid feed during weaning. Prior to weaning, the dominant bacterial families appear to be Bacteroidaceae and Veillonellaceae. However, on day 26, following weaning, the predominant family was Lactobacillaceae (31.4%) with *Lactobacillus amylovorus* identified as the predominant species within this group. Previous studies have also observed an increase in *L. amylovorus* following weaning (Janczyk et al., 2007; Pieper et al., 2008). Other bacterial families with high representation at weaning were Lachnospiraceae (13.4%), Ruminococcaceae (13.2%), and Enterococcaceae (2.7%).

At the end of the nursery period (day 61), typical diets change with the removal of animal proteins and addition of more plant proteins. This dietary change resulted in the family Streptococcaceae (28.8%) being the dominant representative, whereas Lactobacillaceae (9.8%), Lachnospiraceae (8.8%), Ruminococcaceae (8.6%), Clostridiaceae (5.6%), Veillonellaceae (4.9%), and Erysipelotrichaceae (3.5%) were observed at much lower abundances. Among Streptococcaceae members, the most significant increase was noted in the genus *Streptococcus*, particularly in the species *Streptococcus alactolyticus* which corroborates previous research (Leser et al., 2002). Interestingly, there was an increase in the amount of *Bifidobacterium* observed in the fecal samples collected on day 26; however, this increase was not observed on day 61. The authors hypothesized that this short-term increase in Bifidobacteria might have been due to the presence of lactose resulting from the incorporation of whey proteins in diets used early in the weaning period (Hinkle et al., 2012).

More recently, De Rodas et al. (2018) conducted a study to evaluate the microbiome of commercial pigs over time (i.e., farrow to finish) and across different sampling locations (small intestine, cecum, and colon). This study included an operational taxonomic unit (**OTU**)-level analysis to identify the most abundant OTUs over time and across sampling locations. Among the top 50 most abundant OTUs, *Lactobacillus* species (e.g., *L. johsoniilgasseri, L. reuteri,* and *L. mucosae*) and OTUs within the class Clostridia (e.g., *Clostridium*) were prominent. This analysis leads to the conclusion that a dominant bacterial community exists throughout the GIT irrespective of sampling location.

The succession of the microbiota which develops in the pig also depends on the types of exposures as well as management practices such as the early-life use of antibiotics. Schmidt et al. (2011) studied the impact of excessive hygiene on the development of the pig microbiota by rearing pigs in high-hygiene isolators after they had initially been allowed to colonize naturally by microorganisms in outdoor and indoor rearing systems. Rearing the animals in high-hygiene isolators resulted in the disruption of the microbial succession and stabilization events that are known to occur in conventionally reared animals. The authors concluded that the early establishment and development of a normal pig microbiota required continuous microbial exposure and that conditions of excessive hygiene interfered with this natural process (Schmidt et al., 2011). Schokker et al. (2014) looked at the impact of early-life exposure to antibiotics as well as stressful management practices on the gut microbial community and on genome-wide intestinal transcriptome profiles. The authors observed that the use of antibiotics early in life altered the composition and diversity of the gut microbiota and reduced the expression of genes related to a number of immune-related processes (Schokker et al., 2014). Studies have also looked at the impact of the early-life pig microbiota on health outcomes later in life. Using fecal samples, Dou et al. (2017) investigated the impact of the early-life gut microbiota on the susceptibility of pigs to postweaning diarrhea using a combination of culture-dependent and culture-independent techniques. The results revealed that as early as postnatal day 7, the diversity and composition of the fecal microbiota discriminated between pigs that remained healthy and pigs that developed postweaning diarrhea. On postnatal day 7, pigs which remained healthy had higher abundances of Lachnospiraceae, Ruminococcaceae, and Prevotellaceae members and lower abundances of the families Fusobacteriaceae and Corynebacteriaceae, compared with the pigs which developed diarrhea (Dou et al., 2017). Therefore, the early-life microbiota of the pig may have an impact on health and disease later on in life and can potentially be used as a biomarker for disease susceptibility.

NUTRIENT EFFECTS ON MICROBIAL ECOLOGY

Although the gut microbiome changes with age, diet (nutrition) has a significant effect on microbial alteration along the GIT. Outside of the lactation and nursery phases, the commercial swine diet only differs in crude protein and fiber content based on production phase, whereas energy, including lipids and starches, is relatively static at approximately 2,500 kcal/kg on a net energy basis (National Research Council, 2012). Postweaning, piglets will see a great diversity of feed ingredients, typically from high protein sources, included in the diet that are easily digestible while facilitating rapid growth of intestinal and body tissues; however, these ingredients are tapered off in favor of corn and soybean meal, and oftentimes, ingredients (e.g., corn co-products) that may be more economical. As the pig grows and maintenance needs increase, protein density of feed decreases in lieu of energy as feed consumption increases to meet amino acid and energy needs to promote maximal growth and tissue deposition. Inclusion of fiber becomes more prominent in gilt developer and gestation diets to provide bulk to the diet when animals are limit fed. As a result, the GIT microbiome is presented with the most prominent flux of nutrients in the lactation and nursery phases with stability and maturation, barring significant health challenges, occurring in the grower periods that follow.

The subsequent sections will provide more detail with respect to protein, lipid, and carbohydrate effects on the pig microbiome. It should be noted, that although it was our intent to review work done in the pig, a gap exists in this area; therefore, research in other species is also included in the sections below. For a summary of the major bacterial genera present in the human microbioime and their associated substrates and metabolites, please see the review paper by Oliphant and Allen-Vercoe (2019).

Protein

Proteins, or more specifically, amino acids, are necessary for tissue deposition and bodily function. Sow's milk is estimated to contain an average of 5.16% crude protein (CP; NRC, 2012). Amino acid requirements in the NRC suggest that piglets start with a requirement of 22.69% CP. Although the amino acid requirements increase with size, crude protein density decreases as AA requirements are met with an increase in feed consumption. By the late finishing phase, the CP requirement drops to 10.41%.

To ease weaning stress and get piglets started on feed, prestarter and early nursery diets contain a diversity of protein sources that are easily digestible to facilitate growth and maturation of the gut and to promote downstream nutrient absorption and growth. Although diets can contain higher CP levels than NRC requirements suggest, too much CP can be concern for causing postweaning diarrhea (Prohászka and Baron, 1980). It has been demonstrated that decreased CP in nursery diets reduced growth performance and resulted in lower plasma urea and ammonia in the digesta with no effect on the microbiome (Nyachoti et al., 2006; Lynch et al., 2009). Wellock et al. (2007) found that higher CP in the diet increased the fluidity and coliform counts of the feces while reducing *Lactobacillus* counts. Similarly, the increase in CP also increased average daily gain (ADG) and better feed conversion efficiency without any effect on average daily feed intake (ADFI). Opapeju et al. (2009) demonstrated similar growth performance results, but after challenging with enterotoxigenic *E. coli* (**ETEC**), it was found that higher CP did not result in advantages in growth response compared with lower CP. In addition, pigs fed the lower crude protein diet had decreased richness and diversity of bacteria in the colon digesta, following challenge with ETEC. Decreasing dietary CP led to a reduction in biogenic amine in the colon while a decrease from 16% to 13% CP resulted in increased expression of occludin, a tight junction protein (Fan et al., 2017). Interestingly, a further reduction to 10% CP reduced expression of occludin, biomarkers for intestinal stem cells, and ileal morphology. Work by Peng et al. (2017) showed that CP reduction did not result in a linear decrease in biogenic amines in digesta from ileum, cecum, or colon, but linear decreases in ammonia, and cecal and colonic short-chain fatty acids (**SCFA**) occurred. Changes in intestinal SCFA production were not affected by reduction in CP shown by Bikker et al. (2006), which may have resulted from using younger aged pigs. Furthermore, although there was no difference in *Lactobacillus* counts between CP levels, *Bifidobacterium* decreased linearly with CP content (Peng et al., 2017). A nursery pig study showed that *Lactobacillus* spp. increased as CP content increased in ileal digesta regardless of protein source (Rist et al., 2014). It is worth noting that changes in *Lactobacillus* counts appear to vary with CP

content and fluctuation may be due to differences in diet, genetics, or even the composition of the initial microbiome.

Comparing the effects of dietary protein sources on the microbiota has been minimally studied. Work by Cao et al. (2016a) leads to the observation that pigs fed soybean meal (**SBM**) and fishmeal had discriminately different microbial profiles compared with cottonseed meal (**CSM**) or SBM–CSM combination. Likewise, fishmeal, SBM, and SBM–CSM diets lead to greater bacterial diversity compared with feeding solely CSM. Interestingly, where the majority of diets resulted in *Firmicutes* as the predominate phyla, *Proteobacteria* was the dominate phyla in pigs fed the fishmeal-based diet. In addition, it was also demonstrated that CSM-based diets had increased abundance of *Lactobacillus* spp. and may be beneficial for intestinal health. On the other hand, fishmeal had increased abundance of *Escherichia* and *Shigella* species demonstrating that fishmeal may promote an environment with increased susceptibility to postweaning diarrhea. However, it is important to note that the diets included in this particular example vary in composition of other nutrients (e.g., fiber) which may contribute to the differences in microbial composition.

It is important to note that the pig diet may vary in protein source and amino acid composition. In addition, digestibility of dietary protein is more variable than carbohydrates or fats (Yao et al., 2016). These factors, coupled with macronutrient ratios and transit time, may result in different amino acid concentrations that are available to gut bacteria potentially affecting microbial composition, fermentation products of those microbes, and ultimately, host health (reviewed by Oliphant and Allen-Verco, 2019).

Lipid

There is evidence from human and animal studies that dietary fat intake affects gut microbial composition (Wolters et al., 2018). In mice models, it has been observed that following consumption of a high-fat diet, there is a decrease in gut microbial diversity and richness (Zhang et al., 2012), and there is an increase in the relative abundance of Firmicutes and a decrease in the abundance of Bacteroidetes (Shang et al., 2017). However, at lower taxonomic levels, results tend to be more variable. Due to energy density, dietary lipids have mostly been a concern as an energy source and less emphasis has been placed on lipid composition and the effects of specific lipids on the gut microbial community. Studies assessing the effect of dietary fat level are generally focused on obesity models (e.g., using high-fat, Western-type diets). Additionally, these models use higher fat inclusions than typically seen in commercial diets. Furthermore, many of these models also incorporate the use of fiber, prebiotics, or other antiobesogenic compounds for determination of weight loss or decreased fat deposition (Yan et al., 2013; Heinritz et al., 2016). Lipid composition, identifying the effects of saturated, monounsaturated, and polyunsaturated fatty acids (PUFA) on the growth and physiology of pigs, has been reviewed elsewhere (Raj et al., 2017). With respect to the effects of dietary fat on gut microbial communities, very little research has been conducted. Due to their potential anti-inflammatory effects, omega-3 fatty acids have also been considered in nutrition studies. Gestation and lactation diets supplemented with PUFA enhanced glucose uptake in intestinal tissue and glycogen storage in weanling pigs (Gabler et al., 2007). Diets with PUFA supplementation appear to decrease intestinal endotoxin transport, endotoxemia, and TLR-4 activation when compared with saturated fat supplementation (Liu et al., 2012; Mani et al., 2013). Omega-3 PUFA appeared to decrease *Bacteroides* species in the cecum of pigs without any effect of diet or microbiome on fat accumulation (Andersen et al., 2011). Pusceddu et al. (2015) reported that omega-3 PUFA may aid in reducing maternal separation stress by altering the microbiome and decreasing the corticosterone response in rats. Tanghe et al. (2015) found higher levels of DHA in the brain tissue of heaviest pigs of litter compared with the smallest, which may be related to higher mortality in low birth-weight pigs. Supplementation of high-fat PUFA decreased abundance of *Streptococcus*, *Clostridium*, and pathogenic *Enterobacteriaceae* while also increasing bacterial diversity in premature infants (Younge et al., 2017). Research utilizing a murine model found that n-6 PUFA induced inflammation prone organisms (e.g., *Clostridia* spp. and *Enterobacteriaceae*), whereas n-3 PUFA suppressed inflammatory organisms while promoting *Lactobacillus* and *Bifidobacteria* (Ghosh et al., 2013). Taken together, n-3 PUFA supplementation may be useful for gut health and modulating the microbiome to favor beneficial commensal organisms over pathogenic bacteria. Because very little research, especially in pigs and with specific lipid components, has been conducted, this area has potential for future research in delineating the effects of dietary fat:microbiome interactions with respect to growth and health of pigs.

Carbohydrates

Carbohydrates included in pig diets range from simple (i.e., mono- and disaccharides) to complex (e.g., prebiotic oligosaccharides and polysaccharides) and include starch and nonstarch polysaccharides, the latter being commonly referred to as dietary fiber. This section will primarily focus on nonstarch polysaccharides.

There are both negative and positive effects of dietary fiber when included in pig diets. Fiber is necessary to maintain normal physiological functions and provides substrate for gut microbes. In young pigs, higher fiber dietary inclusion promoted *Lactobacillus* abundance in the small intestine and volatile fatty acid (VFA) formation in the hindgut (Bikker et al., 2006). Diets devoid of fiber or just containing prebiotic carbohydrates were found to dramatically shift the microbiome of mice by promoting mucus-degrading bacteria resulting in a decreased mucus thickness of the colon while increasing susceptibility to *C. rodentium,* an enteric pathogen in mice, leading to increased shedding, weight loss, and death (Desai et al., 2016).

A recent report has suggested that insoluble fiber content from a 30% DDGS diet not only shifts the microbiome by reducing the *Firmicutes:Bacteroidetes* ratio and *Lactobacillus* abundance, but this reduction along with alterations in the metabolome may leave pigs susceptible to colitis (Burrough et al., 2015). When using fiber derived from different grain products in nursery diets, Chen et al. (2013) found that wheat bran fiber increased villus height and villus:crypt depth ratio in the ileum and ileum and colon Goblet cell number compared with maize and soybean fiber. Soybean fiber resulted in higher colon VFA concentrations relative to maize fiber, but similar VFA concentrations were noted in wheat bran and pea fiber diets. Abundance of *Lactobacillus* and *Bifidobacterium* appeared to increase due to pea, maize, and wheat bran fiber inclusion, but decreased in control and soybean fiber diets. Wheat bran fiber diets reduced *E. coli* counts in ileal and colonic digesta, whereas soybean fiber had increased numbers of these bacteria. Wheat bran fiber also increased transcription of tight junction proteins zonula occludens 1 and occludin, and TLR2, which could translate to improved barrier function. A follow-up study by Chen et al. (2014) with grow-finish pigs found that pea fiber increased villus height in the jejunum and ileum and ileal sucrase and maltase production compared with soybean fiber. In addition, wheat bran improved ileal villus:height ratio and sucrose production when compared with soybean fiber. Soybean fiber inclusion promoted acetate production in both the ileum and cecum when compared with the control diet although no difference was observed in total VFA production. Wheat bran inclusion resulted in increased cecal butyrate compared with all treatments, suggesting potential for improving cell tissue health similar to data reported by Molist et al. (2009). Interestingly, alfalfa and pure cellulose were shown to increase gene expression for butyrate production in the lumen of the cecum, but not the mucosa when compared against wheat bran (Mu et al., 2017). Alfalfa increased total VFA in the proximal colon compared with wheat bran, but was not different in the cecum or distal colon.

Similar to their previous data, Chen et al. (2014) found that pea fiber and wheat bran resulted in a numerical increase in ileal *Bifidobacteruim*, and an increase in *Lactobacillus* while decreasing *E. coli* concentrations with the opposite occurring in soybean fiber. Similar trends were noted in the colonic digesta. Pea fiber increased jejunal glucose transporter 2 gene expression compared with the control and soybean fiber diets. Pea fiber and wheat bran both increased ileal glucagon and glucose transporter 2 gene expression over maize and soybean fibers, whereas only wheat bran improved sodium-glucose–linked transporter expression.

Molist et al. (2009) found that insoluble fiber from wheat bran had less unbound water in the colonic digesta compared with control or sugar beet pulp diets suggesting higher water binding capacity, allowing for increased substrate for large intestine microflora. Milled wheat bran was shown to increase fecal score and *E. coli* concentration while decreasing total VFA and acetic acid production compared with coarse wheat bran after being challenged with *E. coli* (Molist et al., 2012). Pigs fed the wheat bran had similar fecal scores to the antibiotic control, reduction in *E. coli*, and an increase in VFA production. Cao et al. (2016b) reported Lantang pigs fed a low-fiber diet had increased methane production compared with pigs fed a high-fiber diet. This methane production was positively correlated with higher density of methanogenic bacteria although the mode of action of rice hulls in the high-fiber diet on decreasing methane production was unclear. Likewise, inclusion of pea fiber increased the diversity of methanogenic bacteria in weanling and finisher pigs (Luo et al., 2017), but it was not reported if this altered methane production.

Overall, nutrients contained in the porcine diet (especially protein, lipids, and carbohydrates) may have profound effects on the porcine microbiota and diet–microbiome interactions affect host health and metabolism. Inasmuch as many of the observed diet effects on microbiome composition are reproducible, more research is warranted examining the effects of diet–microbiome interactions on specific host phenotypes in the pig.

PREBIOTICS, PROBIOTICS, AND SYNBIOTICS

During the past 2 decades, prebiotics and probiotics have been investigated to improve the microbial community of the GIT, which confers benefits upon gut health, immune status, and eventually animal growth and health (Gaggia et al., 2010). A balanced gut microbiota is critical to the intestinal structure and function of the host. Obviously, there are concerns relative to antibiotic resistant bacteria. There are many factors associated with a balanced and health promoting gut microbiota, and prebiotics, probiotics, and synbiotics represent potential alternatives to growth-promoting antimicrobials that may maintain or improve animal health. The objective of this section was to provide a few examples of current achievements of utilizing prebiotics and probiotics in weanling and disease-challenged pigs. Comprehensive reviews of prebiotic, probiotic, and synbiotic feed additives are available elsewhere (Guevarra et al., 2019; Barba-Vidal et al., 2018; Liu et al., 2018).

Prebiotics

As functional foods have been well-defined (German et al., 1999), the concept and practice of including prebiotics to pig diets as additives that may affect the GIT microbiota and gut health has increased. A prebiotic is defined as "a selectively fermented ingredient that allows specific changes, both in composition and (or) activity of the gastrointestinal microbiota that confers benefits upon host well-being and health" (Gibson et al., 2004). According to this definition, resistant starch, nonstarch polysaccharides, unabsorbed sugars, and oligosaccharides have been investigated as a source of prebiotics during the past 2 decades. To date, fructo-oligosaccharides, galacto-oligosaccharides (**GOS**), and mannan-oligosaccharides (**MOS**) have shown beneficial effects on gut bacterial community and growth and health of pigs (Zhao et al., 2012; Zhao et al., 2013; McDonnell et al., 2016). The prebiotic potential of resistant starch (**RS**) has been recently studied; nevertheless, no clear evidence of promotion of bacterial structure or improvement of growth performance has been observed (Heo et al., 2014; Sun et al., 2015). It is uncertain whether chito-oligosaccharides (**COS**) can be used as a prebiotic, as the effects of COS in growth performance and intestinal morphology showed inverse results between 2 nursery studies (Liu et al., 2008; Xiong et al., 2015). On the other hand, studies on seaweed-derived polysaccharides (i.e., laminarin and fucoidan; sea-weed extract; **SWE**) have shown encouraging outcomes in pigs challenged with pathogenic bacteria (Heim et al., 2014; McDonnell et al., 2016).

Prebiotics in gut health. As mentioned previously, prebiotics modulate gut health primarily through alteration of the intestinal microbial community. Dietary fructan and COS altered bacterial community by decreasing bacterial counts of *E. coli* and increasing *Lactobacillus* populations (Liu et al., 2008; Zhao et al., 2013). In a *Salmonella*-challenge study, dietary GOS increased intestinal *Lactobacillus* spp. on day 17 post-challenge (MacDonnell et al., 2016). Supplementation of SWE reduced Gram-negative bacteria such as pathogenic *E. coli* and *Salmonella typhimurium* in pigs (Heim et al., 2014; McDonnell et al., 2016). Prebiotics have been reported to increase intestinal SCFA, the major metabolic products of bacterial fermentation (Loh et al., 2006). Metzler-Zebeli et al. (2010) suggested that the functional properties of prebiotics depend on the ileal flow of fermentable substrates. The increased viscosity of nonstarch polysaccharides reduced butyrate production and increased occurrences of *E. coli* virulence factors. Additionally, improved gut morphological structure and decreased incidence of postweaning diarrhea have been reported in pigs supplemented with dietary prebiotics (Liu et al., 2008; Zhao et al., 2012; Heim et al., 2014).

Prebiotics in immunity. The effects of dietary prebiotics on immune responses are contradictory. For instance, Mukhopadhya et al. (2012) did not observe differences in cytokine expression in growing pigs fed SWE. Similarly, the serum immunoglobulin G (**IgG**) concentration and blood profiles were not affected by feeding fructooligosaccharides and MOS (Zhao et al., 2012). In contrast, maternal supplementation of SWE down-regulated the expression of interleukin (**IL**)-6 mRNA in their offspring not challenged with *E. coli* K88, but this difference

was not observed in the challenged piglets (Heim et al., 2014). In a *Salmonella*-challenged growing–finishing pig study, dietary SWE and GOS benefited the immune responses by reducing proinflammatory cytokines (IL-22, IL-6, and tumor necrosis alpha), whereas supplementation of GOS also increased anti-inflammatory cytokine IL-10 and mucin 2 (McDonnell et al., 2016). Recently, resistant starch has been investigated for prebiotic effects. However, increased pro-inflammatory cytokine IL-1β was reported, indicating a negative impact of resistant starch on the immune system (Sun et al., 2015). These results suggest that the effects of prebiotics on immune status depend on many factors, such as the prebiotic source, age of pigs, feeding strategy, health condition, and source of disease challenge.

Prebiotics in growth and digestibility. Feeding prebiotics typically shows moderate beneficial effects on growth performance and digestibility estimates. Liu et al. (2008) reported improved ADG, ADFI, and feed efficiency as well as apparent digestibility estimates in weanling pigs fed dietary COS, whereas others suggested reduced growth performance by feeding COS (Xiong et al., 2015). Feeding MOS increased ADG and ADFI of weanling pigs, which was likely contributed by the increased digestibility of dry matter (DM) and N, whereas fructan did not affect growth performance of pigs (Zhao et al., 2012). In finishing pigs, however, dietary fructan increased ADG, feed efficiency, and digestibility of DM and gross energy (GE; Zhao et al., 2013). Maternal supplementation of SWE improved growth performance of weanling pigs challenged with *E. coli*, but this beneficial effect was not observed in unchallenged pigs (Heim et al., 2014).

Probiotics

Probiotics are nonpathogenic microbes that are able to colonize in the intestines or pass through the GIT in abundant populations to grant benefit to the host (de Vrese and Schrezenmeir, 2008). In pigs, *Lactobacillus* species, *Bifidobacterium*, *Enterococcus faecium*, and *E. coli* have been developed as probiotics to promote mucosal immunity and epithelial function and inhibit growth of pathogenic bacteria (Setia et al., 2009; Lahteinen et al., 2010; Klingspor et al., 2013). However, the probiotic effects rely on the specific bacterial isolates and are inconsistent across studies (de Lange et al., 2010). For example, direct-fed microbial mixtures in *Salmonella*-changed weanling pigs showed little or no beneficial effects on growth performance, immune status, and intestinal morphological measurements (Walsh et al., 2012b, a), whereas others showed improved immune response by feeding *Bacillus cereus* (Scharek-Tedin et al., 2013). In addition, piglets from maternal supplementation of probiotic showed improved gut morphology (Siggers et al., 2008). How to determine a probiotic species and maintain the viability in vivo have been the crucial and demanding issues in the development and application of probiotics (de Lange et al., 2010). The following includes discussion on the fundamental principles of probiotic effects relating to host health.

Probiotics in intestinal barrier function. The intestinal epithelium is in direct contact with the luminal digesta and gut microflora. To prevent uncontrolled inflammatory responses, the mucous layer, in addition with antimicrobial peptides, secretory IgA, and the epithelial tight junctions, plays an important role as the intestinal barrier defenses (Ohland and Macnaughton, 2010). The effects of probiotics on intestinal barrier defenses have been reviewed previously by Ohland and MacNaughton (2010). Briefly, the mucous layer is mostly abundant in glycosylated proteins (i.e., mucin 2) synthesized by goblet cells, which function in proteolytic resistance. The NH_2- and COOH-termini of glycoproteins provide hydrophilic capacity to the mucous layer; some are rich in cysteine residues that form the backbone of the mucous layer via disulfide bonds. This allows the mucous layer to act as the first shield to prevent pathogenic bacteria from reaching the epithelial cells. Probiotics may improve intestinal barrier function by promoting mucus secretion, producing antimicrobial factors (i.e., SCFA, bacteriocins, and lowered luminal pH), and competing for binding sites along the epithelium (Ohland and MacNaughton, 2010). For instance, supplementation of *Enterococcus faecium* facilitated the absorptive and secretory capacity of piglet jejunum, indicating improved intestinal barrier properties (Klingspor et al., 2013). In an in vitro study, *Lactobacillus mucosae* suppressed the adhesion of pathogenic *E. coli* and *S. typhimurium* to pig ileal mucin (Valeriano et al., 2014). Probiotics confer beneficial effects to tight junction integrity, for disrupted tight junctions is associated with increased epithelial permeability, resulting in acute inflammation (Ulluwishewa et al., 2011). Administration of *Lactobacillus plantarum* decreased human epithelial permeability by inducing translocation of tight junction proteins (Karczewski et al., 2010). Butyrate, as the major bacterial metabolite, was

reported to improve the intestinal barrier by accelerating tight junction assembly (Peng et al., 2009). Additionally, reduced permeability of the intestinal epithelium may contribute to the decreased incidences of diarrhea in pigs fed probiotics (Zeyner and Boldt, 2006).

Probiotics in immunity. As previously mentioned, secretory IgA is a major component of the intestinal barrier defenses, which binds to antigens on the surface of pathogens to prevent the intestinal epithelium from colonization and (or) invasion by lumenal bacteria (Ohland and MacNaughton, 2010). Oral administration of *Lactobacillus casei* in mice increased IgA-producing cells, indicating a positive effect on the humoral immune response (Galdeano and Perdigon, 2006). Fecal IgA was increased in sows fed *Bacillus cereus* and their piglets, whereas *Enterococcus faecium* did not affect fecal IgA (Scharek et al., 2007); however, concentrations of circulating IgG were decreased in piglets from both probiotic groups, indicating that the improved mucosal defense resulted in decreased IgG production in piglets (Scharek et al., 2007). In contrast, Lan et al. (2016) reported that feeding a mixture of probiotics had no affects on circulating white blood cells and lymphocytes in weanling pigs. Arpaia et al. (2013) suggested that the commensal bacteria may interact with host immune system via bacterial metabolites, as they found that administration of butyrate and propionate facilitated differentiation of regulatory T cells in mice.

The effects of prebiotics in digestibility estimates and growth performance have been inconsistent, especially in healthy pigs, as other dietary components may have contributed to prebiotic compounds (Gaggia et al., 2010). Probiotics directly promote gut barrier function and communicate with the host immune system through bacterial metabolites. In summary, although contradictory results exist in prebiotic and probiotic feeding trials, more research is warranted to investigate standardized feeding strategies for application of prebiotics and probiotics in healthy and sick pigs. Future studies could also target potential combinations of appropriate prebiotics and probiotics (i.e., synbiotics) to improve gut ecological balance and to reduce the risk of intestinal disorders.

FUTURE OF MICROBIOME RESEARCH—OPPORTUNITIES AND CHALLENGES

With the advancement of next generation sequencing technology and the decrease in cost of sequencing, studies investigating the composition and the dynamics of the pig microbiome has increased (Isaacson and Kim, 2012; Choy et al., 2014; Mach et al., 2015; Pajarillo et al., 2015; Espinosa-Gongora et al., 2016; Heinritz et al., 2016; Sun et al., 2016; van Sambeek et al., 2016; Xiao et al., 2016; Yang et al., 2016; De Rodas et al., 2018; Liu et al., 2018; Tran et al., 2018). These studies have provided valuable information into the compositional changes and associations between the porcine gut microbial communities and production traits (He et al., 2016; Ramayo-Caldas et al., 2016; Yang et al., 2016; McCormack et al., 2017). However, to understand the therapeutic potential of the gut microbiome and to perform "microbiome engineering" (Foo et al., 2017) to improve animal health and performance, we need to look beyond compositional changes and understand causative effects of the microbiome. Partly, the reason for limited number of studies investigating the functional role of the pig and other livestock microbiomes is computational and bioinformatics bottlenecks, as less reference genomes from livestock microbiomes are available for annotation and further analysis. However, with the advancements of bioinformatic tools in recent years for human microbiome investigations (Saeed and Halgamuge, 2009; Waldram et al., 2009; Karlsson et al., 2011; Borenstein, 2012; Greenblum et al., 2012; Zhao et al., 2012; Albertsen et al., 2013; Bisanz et al., 2014; Twardziok et al., 2014; Borenstein, 2015; Seah and Gruber-Vodicka, 2015; Tao et al., 2015; Frank et al., 2016; Sedlar et al., 2017; Beaulaurier et al., 2018; Oliveira et al., 2018), a deeper understanding of the functional role of livestock microbiomes can be achieved. To this end, future research can take many forms to better understand the pig and other livestock microbiomes and to develop novel technologies to improve animal health and performance.

Genome-Centric Approaches for Microbiome Studies

With decreased sequencing costs, investigators have begun to perform shotgun metagenome sequencing to understand potential metabolic functions of the pig microbiome (Ramayo-Caldas et al., 2016; Xiao et al., 2016; Tan et al., 2017; Yang et al., 2017). However, many of these investigations have used gene centric approaches where distribution of gene composition and abundance has been evaluated. However, such approaches only use a small proportion of the data set and provide limited information into the role of the microbiome in animal health and performance. Conversely, recent

developments in bioinformatic approaches have developed new algorithms to assemble microbial genomes using shotgun metagenome data sets with the help of single copy genes, read abundance, and tetranucleotide frequencies (Sangwan et al., 2016; Bowers et al., 2018; Parks et al., 2018; Stewart et al., 2018; Tully et al., 2018). These new tools can help move beyond gene-centric and compositional changes to investigate genome-centric structure functional relationships within the microbiome. Additionally, such approaches are database-independent and as such increase baseline information of microbiome function enabling increased annotation of shotgun reads in future studies.

In addition to genome binning, single cell genomics is another strategy to perform genome-centric analysis of microbiomes. Single cell genomics, although not a new concept (Tolonen and Xavier, 2017), hold great promise in understanding structure-function relationships, identifying rare populations and identifying organisms that are currently unculturable. Such approaches, together with binning approaches identified above, can have the potential to investigate microbiome subpopulations with great efficiency. Additionally, such genome-centric approaches will provide organism-based metabolic information and will provide opportunities to identify microbe–microbe interactions and phage–microbe interactions to better understand ecosystem function and efficiency providing valuable information into methods and opportunities for "microbiome engineering" (Foo et al., 2017) in the future.

Investigating the Microbiome as a "system"

To date, most microbiome studies in livestock species have used gene-centric approaches to investigate the microbiome, which has resulted in a catalogue of microbiome components and functions. However, this "reductionist" approach (Layeghifard et al., 2017) limits the understanding of the microbial community that works as a single entity or system to increase ecosystem function. Therefore, future investigations need to utilize system biology approaches to investigate the microbiome as a whole to understand factors and processors that help shape the microbiome. System biology approaches have already been utilized in human microbiome investigations (Karlsson et al., 2011; Borenstein, 2012; Greenblum et al., 2012; Layeghifard et al., 2017). However, in the livestock industry, system-based approaches are rarely used for microbiome analyses. Utilizing such approaches provide new insight into assembly, adaptation, organization, and functional role of the microbiome and may provide insight into in vivo selection approaches to develop novel direct-fed microbials, and to better understand the factors that influence the gut microbome in pigs and other livestock species. Currently, network-based approaches are used to investigate microbial interactions, yet the potential of using system-based approaches to analyze the microbiome has greater potential to link microbiome to host phenotypes and to develop predictive models. However, to develop predictive models based on microbiomes, phenotypes associated with each microbiome need to be collected. As such, future microbiome investigations need to collect many types of phenotypic and "omic" data including transcriptomic, host genomic, and metabolomic information (Waldor et al., 2015). Such information can be used to develop mechanistic models to help go beyond correlations/associations identified in most current studies.

Understanding the "players" and Opportunities Using the Pig

As stated above, many studies have investigated microbiome composition in livestock species including the pig; however, our understanding of microbial persistence, co-evolution of host microbe interactions, and microbial adaptation is limited. What allochthonus and autochthonus microbial species reside in pigs, what features do these microbes possess to persist in this environment? Why/how does microbial populations fluctuate and what is the consequence of such fluctuations? This line of questioning is critical to understand what evolutionary and environmental forces shape the microbial community and its function within the host. Such information may help understand if host factors or microbial assemblages help microbial persistence. To this end, utilizing a gnotobiotic pig model may provide unprecedented information into microbial assembly, persistence, and host microbe interactions. The gnotobiotic pig model has been used to investigate the human microbiome (Zhang et al., 2013; Wang and Donovan, 2015; Aluthge et al., 2017) due to its similarity to humans in terms of genetics, anatomy, and physiology (Hart et al., 2007). Additionally, pigs have been used as natural and experimental models for many human infectious diseases. Infectious diseases such as *Staphylococcus aureus* (Luna et al., 2009), *Bordetella pertussis* (Elahi et al., 2007), *Mycobacterium tuberculosis*

(Gil et al., 2010), *Pseudomonas aeruginosa* (Luna et al., 2009), *Cryptosporidium parvum* (Argenzio et al., 1990), *Helicobacter pylori* (Nedrud, 1999), hepatitis E virus (Krawczynski et al., 2011), rotavirus, and norovirus (Meurens et al., 2012) have been investigated using pig models. As such, a gnotobiotic pig model will help better understand the causative role of the microbiome and to develop novel probiotic treatments. Additionally, the gnotobiotic pig model can help evaluate direct-fed microbials and their effectiveness in human and pig applications.

Nutrition and Health

With increased understanding of the important role of the microbiome in nutrition and health and the well-documented fact that dietary factors can be used for microbiome manipulation, functional foods or food components can be used as a mechanism to affect the gut microbiota to improve animal health and productivity. As such, future research efforts focusing on "nutritional requirements of the microbiome" and how such dietary factors influence the microbiome will be critical. This line of research may provide insight into feeding pigs and other livestock species under different physiological and metabolic conditions and during poor health, providing opportunities for "precision nutrition" for livestock species. Therefore, in order to narrow the path towards more meaningful porcine microbiome research, future experiments will need to focus on microbe–phenotype relationships (Surana and Kasper, 2017) to better harness the power of the microbiome to develop new strategies for pork production systems.

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