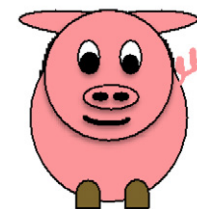


The role of gut microbiota in the health and disease of pigs



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Implications

- The gut microbial ecosystem is fundamental in proper nutritional, physiological, and immunological functions of the pig. However, composition and function of a healthy microbial ecosystem have yet to be qualitatively and quantitatively defined to be used as a tool to maximize animal health and performance.
- As efforts are made to reduce antibiotic use in swine production, the gut microbiota needs to be recognized for its capacity to enhance disease resistance.
- Increasing diet complexity, by including cereal grains high in fermentable carbohydrates, is a sustainable option to increase microbial diversity and beneficial microbes, help prevent incidence of post-weaning diarrhea, and decrease sub-therapeutic antibiotic use.
- Although currently limited by regulatory issues, the introduction of microbial communities that have evolved to protect the pig may increase diversity of the gut microbial ecosystem and provide further protection.

Key words: disease prevention, microbial ecosystem, prebiotics, probiotics, swine

Introduction

The pig intestine is home to a dynamic microbial population that forms a complex ecosystem and has a symbiotic relationship with the host. The population of gut microbes, or microbiota, plays key roles in maintaining nutritional, physiological, and immunological functions of the pig (Lee and Mazmanian, 2010; Brestoff and Artis, 2013). However, this microbial population also includes disease-causing organisms such as *Escherichia coli*, *Salmonella*, and Clostridia. These pathogenic organisms can cause substantial morbidity and mortality; thus, prophylactic use of antibiotics has been common in livestock industries. Disturbances in the gut microbial ecosystem during the rearing of pigs can dramatically increase risk of disease.

To meet production goals in the swine industry, piglets are weaned early, before a stable microbial population is established and the immune system is mature. Stress at weaning further disrupts the gut microbial ecosystem (Konstantinov et al., 2006), increasing susceptibility to bacterial post-weaning diarrhea (Lallès et al., 2004). The use of prophylactic antibiotics tends to balance these disturbances, decreasing incidence of gastrointestinal disease, particularly at weaning. Widespread use of prophylactic and growth-promoting

antibiotics has increased selective pressure for anti-microbial resistant bacteria and is now a major public health concern (Dibner and Richards, 2005). Using current knowledge of the host–microbial relationship, strategies including the use of prebiotics, highly fermentable carbohydrate cereal grains, probiotics, and (or) microbial transplants may promote animal health and reduce the need for antibiotic use. However, future research must quantitatively and qualitatively define the composition and function of a ‘healthy’ pig gut microbiota to successfully implement such strategies.

Important Roles of Microbes

Initial colonization

Microbial colonization of the piglet gut begins immediately following birth. Initial colonization by *E. coli* and *Streptococcus* spp. creates an anaerobic environment for subsequent colonizers, *Bacteroides*, *Bifidobacterium*, *Clostridium*, and *Lactobacillus* (Konstantinov et al., 2006; Petri et al., 2010). The microbes that actually colonize depend on exposures, including the sow and all aspects of the piglet’s environment. The colonization depends not only on the microbes that are introduced, but also their timing, as repeated exposures during development result in a different microbiota than a single exposure of the same inoculum (Schmidt et al., 2011). A known beneficial microbe, *Lactobacillus*, predominates the small intestine of piglets until weaning and is a major player in disease prevention (Konstantinov et al., 2006; Petri et al., 2010). Introduction of cereal-based diets at weaning causes dramatic shifts in the microbiota (Mach et al., 2015). Clustering of piglets toward a specific microbiota type post-weaning, denoted by *Prevotella* abundance, has been associated with increased growth rates (Mach et al., 2015). Future research must focus on how initial colonization and temporal changes during weaning contribute to disease prevention, piglet health, and growth performance long term.

Gut structure and barrier function

The gastrointestinal tract is a multi-function organ, maintaining regular nutrient, water, and electrolyte absorption while acting as a barrier to exclude pathogens and toxins. Using germ-free pigs born and raised in the absence of microbes (Shirkey et al., 2006; Willing and Van Kessel, 2009), microbes were established as playing important roles in disease prevention by developing and maintaining proper gut structure and immune function (Lee and Mazmanian, 2010; Brestoff and Artis, 2013). While germ-free pigs are not realistic for a production system, such models have revealed that microbes colonizing the gastrointestinal tract do not impact development equally (Willing and Van Kessel, 2007; Shirkey et al., 2006). Thus, variation of exposures impacts the functional development of the gastrointestinal tract in many regards.

A single layer of intestinal epithelial cells and specialized proteins between these cells provide a physical barrier to pathogen entry. To maintain a barrier, the intestinal epithelium must be supplied constantly with energy for regeneration. Interactions with the intestinal microbes impact the replacement rate of this cell population and thus impact growth efficiency (Willing and Van Kessel, 2007). One major symbiotic function of the gut microbiota is their ability to provide energy to the intestinal epithelium as short-chain fatty acids (SCFA) by fermenting carbohydrates that are not otherwise digestible by the animal (Sakata, 1987). The SCFA butyrate is the preferred energy substrate for intestinal cells, promoting normal proliferation and differentiation. Dietary inclusion of fermentable carbohydrates is one strategy to promote butyrate-producing bacteria and is the basis for prebiotics (discussed below).

Restricting pathogens from adhering to the intestinal epithelium is another line of defense against disease progression. Mucin production by the intestinal goblet cells creates a dense mucus layer that is impermeable to pathogens and toxins (Jacobi and Odle, 2012). Increasing beneficial microbes, such as *Lactobacillus*, increases mucin production, which improves the gut barrier (Che et al., 2014).

Immune system development

Germ-free animals have major shortcomings in their intestinal and systemic immune systems (Lee and Mazmanian, 2010); therefore, they do not represent a realistic model. However, simply changing the balance of microbes in early life has substantial impacts on autoimmune and inflammatory diseases later in life (Russell et al., 2012, 2015). Mucosal immunoglobulins, IgA, are stimulated by microbial fermentation and limit pathogen entrance through the intestinal epithelial cells (Che et al., 2014). Secretory IgA concentrations are positively correlated with *Prevotella* abundance and increased animal growth (Mach et al., 2015). Similar to the impacts on long-term microbial colonization, continued microbial exposure during piglet development is important for balancing the immune cell population (Inman et al., 2010).

Impact on feed efficiency

Microbial populations may contribute to the efficiency of feed utilization in pigs. The microbial population of pigs differs between efficient and inefficient animals (efficient animals have more *Lactobacillus* spp.). The mechanism involved is not clear; however, more efficient pigs were less responsive to immune

challenge, supporting a theory that increased efficiency results from a dampened innate immune response (Vigors et al., 2016).

Challenges

Dysbiosis and post-weaning diarrhea

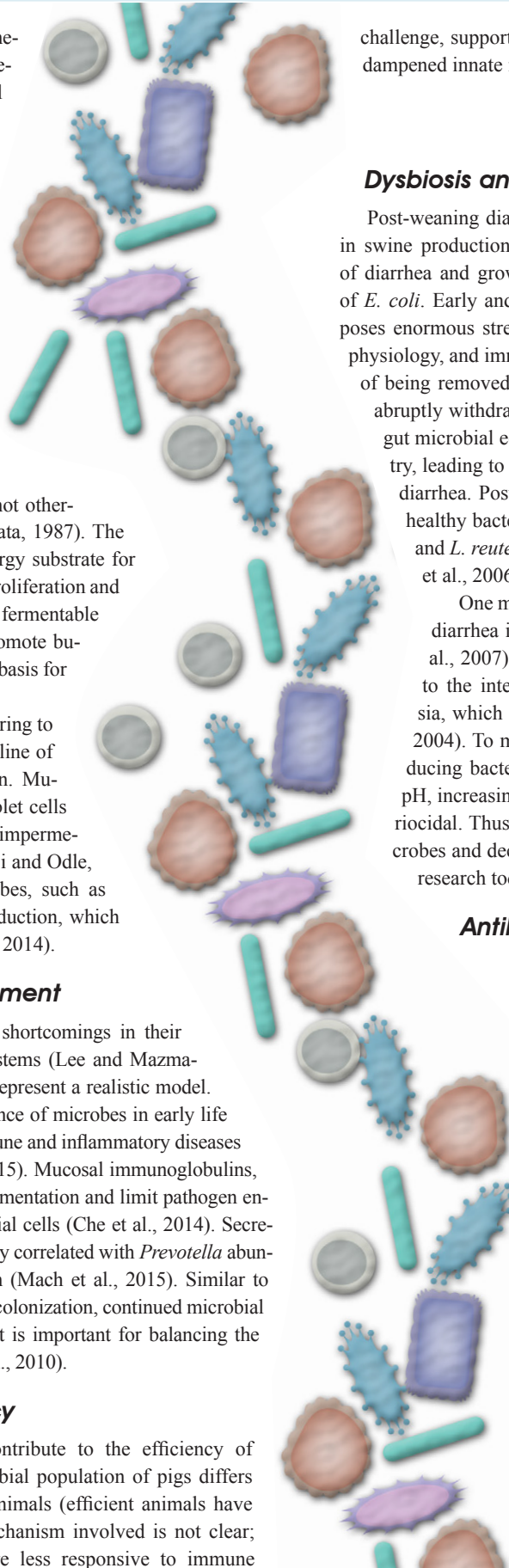
Post-weaning diarrhea is one of the largest sources of economic loss in swine production worldwide. It is characterized by high incidences of diarrhea and growth reduction attributable to enterotoxigenic strains of *E. coli*. Early and abrupt weaning, between 21 and 28 d of age, imposes enormous stress, leading to perturbations in gut microbiota, host physiology, and immune function (Konstantinov et al., 2006). The stress of being removed from the sow, mixed into a new environment, and abruptly withdrawn from the sow's milk is associated with a volatile gut microbial ecosystem and lowered defenses against pathogen entry, leading to increased risk of disease, in particular, post-weaning diarrhea. Post-weaning diarrhea is characterized by reductions in healthy bacteria, including *Lactobacillus sobrius*, *L. acidophilus*, and *L. reuteri*, and increases in pathogenic *E. coli* (Konstantinov et al., 2006; Lallès et al., 2007).

One major factor leading to the dysbiosis and post-weaning diarrhea is low feed and water intake post-weaning (Lallès et al., 2007). This period of anorexia leads to structural changes to the intestine, including villus atrophy and crypt hyperplasia, which further lead to gut barrier dysfunction (Lallès et al., 2004). To make matters worse, the reduction of lactic acid-producing bacteria (*Lactobacillus*) during weaning raises intestinal pH, increasing disease susceptibility because low gut pH is bacteriocidal. Thus, dietary strategies aiming to increase symbiotic microbes and decrease opportunistic pathogens are at the forefront of research today (Lallès et al., 2007; Kim et al., 2012).

Antibiotic use

After the initial discovery of antibiotics to treat disease, low levels of antibiotics (sub-therapeutic) in feed were revealed to increase growth rates of weaned pigs (van der Fels-Klerx et al., 2011; Heo et al., 2013). This has been a cheap and effective way to maximize efficiency; however, widespread antibiotic use in livestock production has likely contributed to the increasing numbers of multi-drug-resistant pathogens; a major human and animal health concern. Although sub-therapeutic antibiotics have proven to improve growth performance, prolonged use may have long-term negative effects on the host, including enriching populations of potential pathogenic bacteria (*Shigella* spp., *E. coli*, and *Salmonella*) that remain long after the initial antibiotic treatment (Janczyk et al., 2007; Schokker et al., 2014).

Sub-therapeutic antibiotics are hypothesized to improve growth through different mechanisms, many of which impact the gut microbial ecosystem; these include: 1) reducing



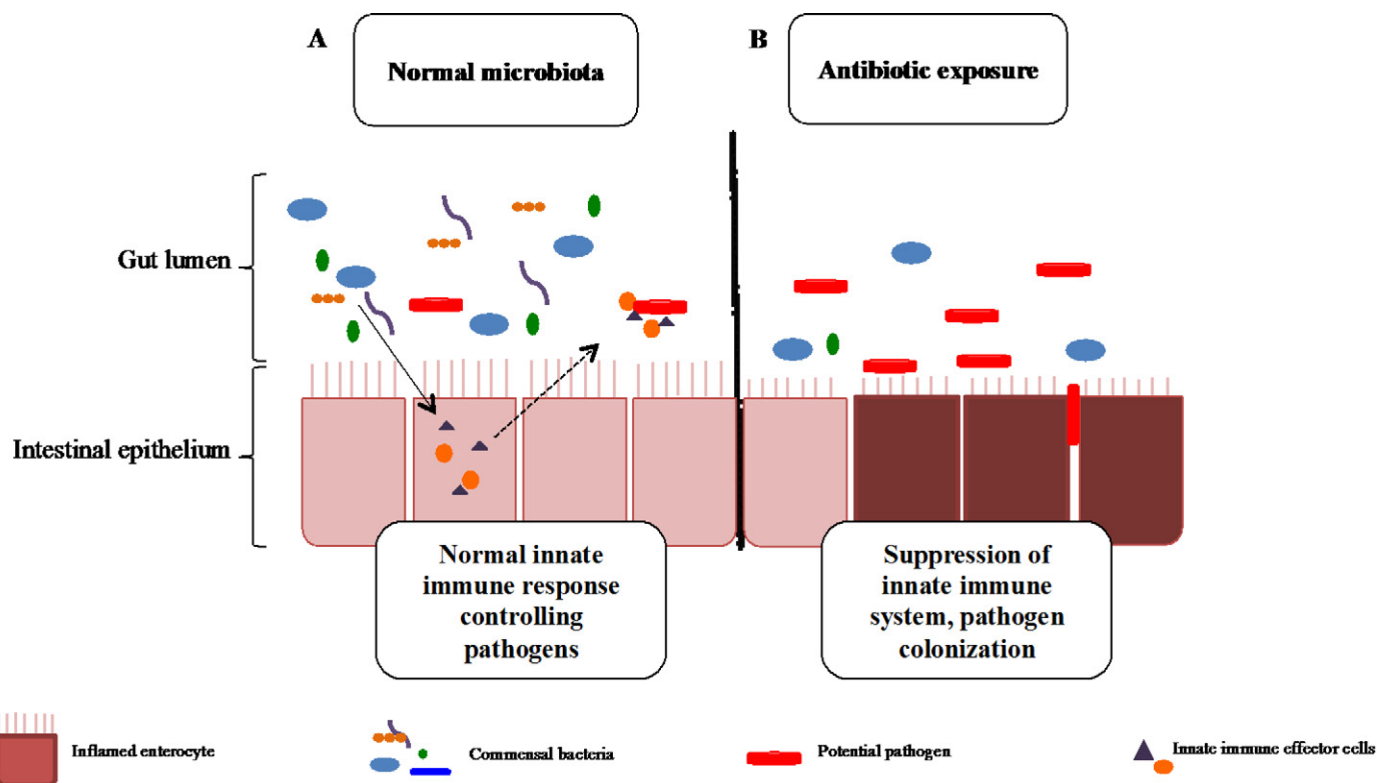


Figure 1. (A) Piglets with normal gut microbiota can detect pathogens and respond with a normal innate immune response controlling pathogen colonization. (B) Exposure of piglets to antibiotics may suppress the innate immune system, leading to increased pathogen colonization and disease susceptibility.

pathogen load, sub-clinical disease, and metabolic cost of immune activation; 2) decreasing growth-reducing metabolites, including ammonia and bile degradation products; 3) decreasing competition from microbes for nutrients; and/or 4) enhancing uptake and use of nutrients due to thinner intestinal wall (Dibner and Richards, 2005; Holman and Chénier, 2015). How sub-therapeutic antibiotics affect the gut microbial ecosystem is somewhat elusive and tends to be antibiotic specific. Commonly used tylosin and a mixture of chlortetracycline, sulfamethazine, and penicillin independently work to reduce *Bacteroidetes* abundance (Looft et al., 2012; Holman and Chénier, 2014). However, administration of carbadox, another common antibiotic, increases relative abundance of *Prevotella*, *Roseburia*, and *Fecalibacterium* (Looft et al., 2014). The functional value of changing microbial taxa with antibiotics is impossible to identify without definition of a 'healthy' gut microbiota. Functionally, antibiotics can increase microbial diversity and downregulate immune genes related to intracellular communication; however, the mechanism through which these changes affect host health, disease resistance, and growth performance remains unknown (Schokker et al., 2014). The downregulation in immune gene expression may allow the host to increase energy recovery through microbial SCFA (Looft et al., 2014). Due to the lack of cohesive evidence on antibiotic function, a fifth alternative hypothesis proposes that antibiotic growth promoters inhibit catabolic mediators of intestinal inflammatory cells, sparing energy for production (Niewold, 2007). Without complete mechanistic understanding of how sub-therapeutic antibiotics work, we must return to a dependence on the commensal microbiota to provide the animal protection. A decline or ban on sub-therapeutic and prophylactic antibiotic use may have serious production consequences as seen during the European Union ban in 2006 (Hayes et al., 2002).

Although minimally studied in pigs, therapeutic doses of antibiotics can increase opportunities for pathogenic organisms to colonize and cause disease (Sekirov et al., 2008). Antibiotic treatment suppresses the host's innate immune defenses, contributing to this increased susceptibility (Willing et al., 2011a; Wlodarska et al., 2011; Menendez et al., 2013), as depicted in Figure 1. Antibiotics will continue to be required for therapeutic treatment of animals; however, the short- and long-term impacts antibiotic treatments have on the microbiota (Janczyk et al., 2007), and thus host resistance, must be taken into account. Efforts to minimize prophylactic group treatment and movement toward individual antibiotic treatment as required will help maintain a healthy herd microbiota.

Food safety

Composition of the pig microbiota is an important determinant of human food safety. The pig acts as reservoir for potential foodborne pathogens, in particular *Salmonella*, which is the leading cause of foodborne illness in humans (Kirk et al., 2015). Pigs carrying *Salmonella* shed the bacteria through their feces, thereby contaminating pen-mates, human food, and the environment. Pigs that shed low or high amounts of *Salmonella* have considerably different gut microbial profiles, indicating specific resident bacteria may protect against *Salmonella* colonization and shedding (Bearson et al., 2013). A connection exists between the gut microbiota and host health, with high-shedding pigs having increased diarrhea post-infection (Bearson et al., 2013) and unique immune gene expression profiles (Huang et al., 2011). High *Salmonella* levels within a swine herd are also associated with reduced growth performance (Farzan and Friendship, 2010), further indicating the important relationship among gut microbial composition, host health, and animal performance.

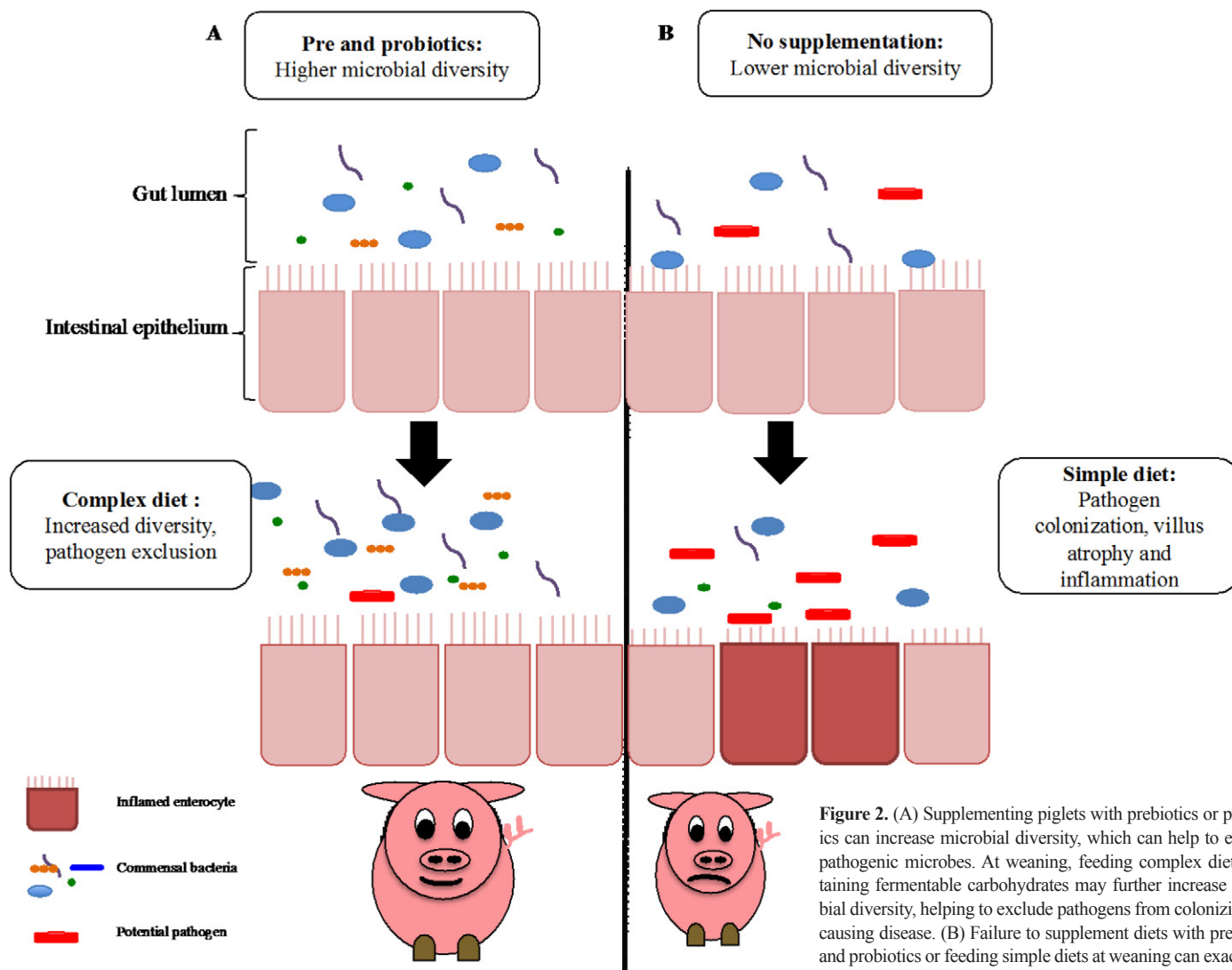


Figure 2. (A) Supplementing piglets with prebiotics or probiotics can increase microbial diversity, which can help to exclude pathogenic microbes. At weaning, feeding complex diets containing fermentable carbohydrates may further increase microbial diversity, helping to exclude pathogens from colonizing and causing disease. (B) Failure to supplement diets with prebiotics and probiotics or feeding simple diets at weaning can exacerbate colonization of pathogens, causing villus atrophy, inflammation, diarrhea, and growth reduction.

Solutions

Strategies to prevent dysbiosis, post-weaning diarrhea, and use of antibiotics focus primarily on promoting a “healthy” gut microbial ecosystem. A major limitation of these strategies is that the definition of a healthy gut microbial ecosystem is not yet achieved.

Prebiotics and fermentable carbohydrates

Coined by Gibson and Roberfroid, a prebiotic is defined as “a selectively fermented ingredient that results in specific changes in the composition and/or activity of gastrointestinal microbiota, thus conferring benefit(s) on host health” (Gibson et al., 2010). In other words, inclusion of prebiotics in swine diets stimulates the proliferation and metabolic activity of beneficial microbes, contributing to a stable microbial ecosystem (Metzler et al., 2005). Prebiotics, such as fructo-oligosaccharides, protect the gut by lowering intestinal pH and increasing cecal and colonic butyrate concentrations (Mikkelsen and Jensen, 2004). Only inulin, fructo-oligosaccharides, transgalacto-oligosaccharides, and lactulose fulfill the old criteria for prebiotic classification although many new “candidate” prebiotics have been recognized (Gibson et al., 2010; Bindels et al., 2015). Dietary carbohydrates,

wheat starch, and beet pulp can also promote microbial fermentation and increase beneficial *Lactobacillus* species in the small intestine (Konstantinov et al., 2004). Bindels and colleagues (Bindels et al., 2015) have proposed a new, more inclusive definition for prebiotics: “a prebiotic is a non-digestible compound that, through its metabolism by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host.” Another limitation to prebiotic use is how the literature largely restricts the definition of “health-promoting bacteria” to only *Bifidobacterium* and *Lactobacillus* (Bindels et al., 2015). In the pig, *Bifidobacterium* constitutes only a small portion of the entire microbiota, indicating that other important microbial taxa or groups of microbes may exist that confer greater benefits to the pig. It is expected, although not proven in the pig, that many bacterial species can act to promote health.

Conventional swine diets are based on cereal grains that naturally contain fermentable carbohydrates, including resistant starch, β -glucans, and hemicellulose. Mutualistic gut microbes can use fermentable carbohydrates within cereal grains as substrates, providing SCFA to the host. The use of naturally occurring, fermentable carbohydrates to promote fermentation and gut health has had documented success (Aumiller et al., 2015; Jha and Berrocoso, 2015).



Piglets suckling represents the last exposure to a mature microbiota.

In the past, swine nutritionists have emphasized including highly digestible cereal grains into weaning pig diets. However, evidence that cereal grains high in fermentable carbohydrates may promote a stable and healthy gut microbial ecosystem is beginning a paradigm shift in formulation practices. Complex diets that replace wheat with barley do increase mucosal microbiota uniformity and growth of piglets after weaning (Levesque et al., 2014). Using fermentable cereal grains may also enhance food safety, with high β -glucan and amylose hull-less barley reducing *Salmonella* persistence and shedding and abundance of *Clostridia* cl I, respectively (Pieper et al., 2012).

Probiotics

Probiotics, known as direct-fed microbials (DFM) in the livestock industry, have long been proposed as alternatives to sub-therapeutic antibiotics. Probiotics are defined as “live microorganisms that, when fed in adequate amounts, confer a health benefit to the host” (Hill et al., 2014). Probiotics can be used to prevent and treat microbial imbalance by altering intestinal populations, epithelial lining, and the gut-associated lymphoid tissues (Metzler et al., 2005). Feeding *Lactobacillus* derived from the pig intestine as probiotics reduced the abundance of Enterobacteriaceae including pathogenic *E. coli*, reduced incidence of diarrhea (Huang et al., 2004; De Angelis et al., 2007; Pieper et al., 2010), enhanced immune response during infection (Naqid et al., 2015), and increased weight gain (Konstantinov et al., 2008). Furthermore, treatment of suckling pigs with *Lactobacillus reuteri* can protect by reducing intestinal pH through lactic acid production via *Bifidobacterium* spp., subsequently reducing abundance of *E. coli* (Hou et al., 2015). *Lactobacillus* probiotics can decrease inflammation, measured as lower expression or serum inflammatory cytokine, which can help divert nutrients toward growth (Qiao et al., 2015). How supplementing prebiotics, probiotics, and complex diets can influence the gut microbiota and subsequent animal performance is depicted in Figure 2. Efficacy of probiotics is inconsistent and typically ceases to work if not applied constantly, with time and dosing playing a major role in their success. Piglets that suckle from probiotic-fed sows may be a promising new development in probiotic application (Scharek-Tedin et al., 2015). Although these piglets had changes in their mucosal immune system (Scharek-Tedin et al., 2015), inconsistency in gut microbial responses from probiotic-fed sows and their suckling piglets leads researchers to hypothesize that response to probiotics differs in each individual (Starke et al., 2013). However, individualized probiotic treatment of sows or their offspring is not a sustainable option in conventional swine operations.

Regulatory issues for direct fed microbials

Although certain probiotics already exist and are in use, there may be other healthy microbes, or groups thereof, that are superior in promoting animal health. In human medicine, increasing evidence exists that our behavior surrounding hygiene in the Western world may be linked to lower gut microbial diversity and a “disappearing microbiota,” which is linked to the increase in immune-mediated diseases (Martínez et al., 2015). Similarly, in intensive livestock systems, we may have inadvertently minimized the exposure of animals to healthy microbes in an effort to reduce pathogen exposure. Thus, going back to a swine’s “native” microbiota may help to protect against pathogens and disease. Discovered in mouse models, fecal microbial transplants can successfully colonize and can increase host resistance to bacterial infection (Willing et al., 2011b). Fecal microbiota transplants are gaining notoriety in human medicine to treat human gut dysfunctions. However, regulatory guidelines within North America may limit their use and development.

Within Canada, the Canadian Food Inspection Agency within the federal Feeds Act and Regulations is responsible for approving new probiotics. However, their safety and efficacy must be evaluated before approval as livestock feed, a veterinary drug, or a biologic. In Canada schedule IV of Feeds Regulations contains only two viable microbial genera (not including yeast or bacterial derived fermentation products) approved for livestock feeds, *Lactobacillus* and *Streptococcus*. Similarly, DFMs are strictly regulated in the US by the Food and Drug Administration, which groups them into three categories, 1) lactic acid-producing bacteria, 2) bacteria belonging to *Bacillus*; and 3) *Saccharomyces* yeast. The idea within Canada and the US that only a few predefined groups of DFMs exist does limit the development of new DFMs that may have different modes of action. To date, regulations do not exist for fecal transplants or development of a microbial bolus that could contain numerous different “healthy microbes.”

Conclusion

The gut microbial ecosystem is essential for normal nutritional, physiological, and immunological functions of the pig. Any disturbance in the microbial ecosystem creates an opportunity for pathogenic organisms to colonize and cause disease. Management practices in intensive swine production including: early and abrupt weaning, extreme hygiene, prophylactic and sub-therapeutic antibiotic use, and diet formulation are unintentionally disturbing the gut microbial ecosystem, predisposing piglets to disease. While the microbiota has been well accepted as an important determinant of disease resistance, a very limited understanding exists of the composition of a healthy gut microbiota in the pig. A basic appreciation for microbial diversity and stability needs to be expanded into microbes that are truly beneficial and fully understood. Concurrently, nutritionists need to shift attention toward feeding the microbiota instead of just the pig. Improving on existing theories of prebiotics and probiotics, inclusion of high-fermentable carbohydrate cereal grains with “prebiotic function” and rethinking regulatory guidelines surrounding DFMs are promising strategies to promote a stable gut microbial ecosystem.

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