

# What Shapes the Piglet Microbiota at Weaning and How it Prepares the Piglet for Disease Challenge

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## Introduction

The number of bacterial, fungal, and archaeal cells residing in the gastrointestinal tract outnumber the somatic cells in the body of the pig. Since shortly after their discovery, antibiotics have been used to control the abundant and diverse bacterial population to increase growth efficiency and reduce disease in an often-untargeted fashion. Over this period extensive antibiotic use has encouraged the development of antibiotic resistance in pathogens and a depletion of the population of microbes that co-evolved to protect the host and shape its physiology (Ley et al. 2008). With increasing pressure to reduce antibiotic use in swine production, there has been an increased effort to understand and take advantage of the microbiota as a key disease resistance mechanism. Research in this area is still in its infancy, with global changes in the microbiota only being associated with health outcomes. For example, the composition of the microbiota pre-infection has been correlated with *Salmonella* shedding in pigs (Bearson et al. 2013). In more controlled rodent studies, it has been shown that resistance to infection with enteric pathogens is transferrable by microbial transplantation

between mice of varying disease resistance (Willing et al. 2011a). However, new resources, such as the reference gene catalogue of the pig gut microbiome (Xiao et al. 2016), in combination with increased concerted efforts to understand the biological basis of host-microbial interaction should lead to rapid advances in the field, as seen in human medicine. Much of the research on the intestinal microbiota of piglets has focused on the effects of dietary and management interventions post-weaning, and only a small amount of research has examined the external factors shaping the microbiota of piglets before and at weaning. This paper outlines some of the established impacts of the microbiota on host health and the environmental factors shaping the composition of the piglet microbiota in the pre-weaning period.

## **Why the microbiota matters**

Unless a piglet is derived by caesarian section and reared in an isolator, microbes will colonize it. However, not all microbes are equal in their ability to provide the beneficial services that co-evolved microbial species do. For example, the T-cell population that is important in preventing excessive inflammation in the intestine (FoxP3+ T-regulatory cells) only establishes properly when gut microbes from the original host species but not gut microbes from another mammalian species colonize (Chung et al. 2012). In human medicine microbes that are efficient at stimulating T-regulatory cell populations have been identified (Atarashi et al. 2013), however, similar studies have not been performed in swine.

Although not clearly defined, early life microbial composition is important to immune development and the long-term composition of the microbiota of pigs. The composition of the microbiota has been associated with many immune parameters, including IgA secretion into the small intestine and development of the T-regulatory cell population, as discussed below. These changes are significant to disease, as rearing piglets with reduced exposure to an adult microbiota resulted in changes in immune development and impaired ability to tolerate infection with *Mycoplasma hyopneumoniae* (Schachtschneider et al. 2013).

In the absence of disease challenge, pigs that lack an immune system will grow more efficiently than those with a functional immune system. This is supported by research correlating the anti-inflammatory properties of oxytetracycline with growth promotion in pigs (Soler et al. 2016). Furthermore, pigs exhibiting a low residual feed intake had immune cells that were less responsive to lipopolysaccharide challenge (Vigors et al. 2016). However, as antibiotics are removed from production systems, it will be important to support pigs with a robust immune system that is able to resist disease, even though it may come with a cost to growth efficiency.

## **Factors shaping the microbiota at weaning**

### ***Antibiotics***

Antibiotics used in swine production, whether therapeutic, prophylactic or sub-therapeutic always target a broad range of bacteria, and never target only a single pathogen of interest. Not surprisingly, this impacts the microbiota in the short term, which also has immediate impacts on innate defense mechanisms (Willing et al. 2011b). However, antibiotic treatment to piglets at processing can impact on the composition of the microbiota weeks later (Janczyk et al. 2007). The injection of Tulathromycin at 4 days of life had substantial effects on the microbiota, which coincided with a significant reduction in many immune-related processes (Schokker et al. 2014). In some cases antibiotic exposure results in transient changes in the microbiota, however the disruption in the microbe-host interaction can have long-term consequences on host defense, such as intestinal alkaline phosphatase activity (Arnal et al. 2014). Furthermore, antibiotic exposure to the mother in late gestation has also been shown to impact on the microbiota of the offspring.

### ***Milk composition and the Nursery diet***

Diet is known to be one of the most important determinants of microbial composition. From birth to weaning the piglet encounters variations in diet including, colostrum intake and subsequent milk consumption, composition of the milk and the formulation of the creep feed. Sow milk is filled with bioactive molecules that are key to the health of the piglet including immunoglobulins, growth factors and antimicrobials. It also contains a diverse set of milk

oligosaccharides that are important for the enrichment of symbiotic bacteria (Salcedo et al. 2016). In humans, milk oligosaccharides are key to the promotion of beneficial *Bifidobacteria* spp. (Coppa et al. 2004). It has yet to be directly tested how variations in porcine milk oligosaccharides affect the composition of the piglet intestinal microbiota, however, initial evidence indicates that bacterial taxa able to use milk oligosaccharides are qualitatively different (Salcedo et al. 2016). There is also relatively little literature examining the effect of nursery diet on microbiota composition. One study has examined dietary fibre source and found potentially beneficial effects of alfalfa inclusion on the microbiota, indicated by a reduction in *Streptococcus suis*, and an increase in generally beneficial *Clostridium* cluster XIVb (Zhang et al. 2016). There are a few studies that have examined the effects of formula feeding and have found substantial differences (Wang et al. 2013), however these studies are likely of little consequence in swine production. Overall, while it likely to be very important to shaping the microbiota at weaning, very little research has explored the effects of piglet diet while on the sow.

### ***The Sow and Environment***

Some studies have explored the effect of sow on the composition of the intestinal microbiota. Even when fostering genetically distant breeds, such as Jinhua and Yorkshire, the microbiota of cross-fostered piglets shifts substantially to resemble the microbiota of step-siblings instead of biological siblings (Xian et al. 2014). Cross-fostered piglets also grew better than biological littermates, suggesting that milk availability or composition of foster sow was likely different. There is

also a substantial effect of the rearing environment. Pigs reared in an outdoor environment have a substantially different microbial community than pigs reared in a clean indoor environment, even though both groups were raised on the sow until weaning (Mulder et al. 2009). Piglets raised indoors had less *Lactobacillus* and higher numbers of potentially pathogenic phylotypes (Mulder et al. 2009). Variations in exposure in the first day of life can persist well beyond weaning. This has been observed for both microbial composition as well as blood metabolites (Merrifield et al. 2016).

### **Sow diet**

The feeding of 3% inulin to sows resulted in changes in the sow microbiota as well as that of the piglets, including some changes that could be beneficial, such as a decrease in enterobacteria and increase in *Clostridium leptum* (Paßlack et al. 2015). The inclusion of short-chain fructooligosaccharide in the sow diet has resulted in increased growth efficiency for piglets on sows that were seropositive for influenza during lactation until 10 weeks of age (Le Bourgot et al. 2016a). Sow scFOS supplementation also increased colostrum IgA content and the secretory IgA production in unstimulated Peyer's patches of the piglets (Le Bourgot et al. 2014). When fed directly to the piglets, the scFOS supplementation increased the piglets ability to mount an anti-influenza IgA response after vaccination (Le Bourgot et al. 2016a). Inclusion of fermentable carbohydrates has been shown to improve sow metabolic health with significant reduction in insulin resistance (Tan et al. 2016), which would impact her microbiota as well as the composition of the milk. Several studies in humans have indicated that

metabolic health and obesity of the mother has substantial impacts on the microbiota of her infant (Soderborg et al. 2016).

### **Sow probiotics**

Although there are limited studies, provision of probiotics to the sow has been associated with improved outcomes in piglets. The inclusion of probiotic *Enterococcus faecium* to the sow during gestation and lactation has also been shown to alter milk composition (increased lactose and decreased somatic cell count with less mCD14+ cells) (Scharek-Tedin et al. 2015). Changes in the sow resulted in altered intestinal microbiota and cytokine expression in the piglets, and the reduction of mCD14+ cells correlated with reduced numbers of activated T cells in the ileal mesenteric lymph nodes of the piglets (Scharek-Tedin et al. 2015). *Bacillus subtilis* used as a direct fed microbial in sows also impacted the piglet microbiome in early life with increases in *Lactobacillus* species and reductions in *E. coli* (Baker et al. 2014). It is clear that different probiotic supplementations have distinct effects on the microbiota of piglets, and further work is needed to establish which are more advantageous.

### **Conclusion**

There is still substantial knowledge to be gained before the characteristics or composition of the ideal microbiota for a piglet at weaning can be defined. There are now a few studies that correlate specific bacterial subsets with production outcomes. For example, the abundance of the genus *Prevotella* has been

associated with IgA production and growth rates (Mach et al. 2015). It is also known that some bacterial populations are typically associated with ill health and intestinal inflammation, such as *Clostridium perfringens* and *Salmonella*. Furthermore, it is now clear that the colonization of the piglet can be modified through management strategies. Now, we just need to know what microbial populations will best support the health of the pig.

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