

7. Microbiota development in piglets

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Abstract

Starting at birth, the intestinal microbiota of piglets develops rapidly from a simple to a more stable, mature ecosystem, following a highly dynamic developmental trajectory that includes a strong disturbance particularly around the time of weaning. The microbiota in the gastro-intestinal tract of piglets plays numerous beneficial roles. Apart from its fermentation activity, the microbiota also stimulates a proper development of gut physiology, host immunity and general well-being of the piglet. Conventional methods to prevent gut-related issues include the use of antimicrobial growth promoters and the inclusion of zinc oxide in feed. As these conventional methods have several drawbacks, new methods are being developed to improve gut microbiota development and thereby prevent gut-related issues. Of these new methods, the inclusion of fibrous components in feed and the administration of probiotics are thought to be the most promising. To date, several *in vivo* studies have shown the effectiveness of including fibrous components or probiotics in feed. Although results are promising in a number of these studies, optimal inclusion rates of fibrous components are still to be found, and the use of probiotics also comes with some practical obstacles. This chapter reviews the available literature on the subject and offers a comprehensive understanding of the factors involved in the use of conventional and innovative methods to ameliorate impaired piglet gut health.

Keywords: gut health, early-life, livestock, prebiotics, probiotics

7.1 Introduction

The gut microbiota has numerous beneficial roles, such as fermentation of fibres, generation of vitamins, stimulation of immune development and prevention of pathogen colonization. To date, the piglet's microbial gut ecology has been extensively studied, and this has been made possible by the advent of molecular, cultivation-dependent approaches, and particularly the more recent rapid developments in next-generation sequencing methods. These methods have allowed several developmental mechanisms to be outlined. One of these mechanisms is that the composition of the microbiota becomes more diverse over time, especially during the first days of postnatal life as microbes from the environment colonize the gastro-intestinal (GI) tract. Starting at birth, the microbiota in the GI tract develops in a highly dynamic environment, with the host gut physiology and the innate immunity developing in parallel (Kim *et al.*, 2012). As in other mammals, the GI tract of piglets is mostly oxidic at birth, although the metabolizing activity of early colonizing microbiota results in oxygen depletion over time during the first postnatal

week. Subsequently, new niches become available for anaerobic microbes, as large parts of the GI tract become anoxic (Jost *et al.*, 2012). This changing environment results in a complex ecosystem with a dynamic composition that shifts over time and along the entire GI tract (Isaacson and Kim, 2012).

Another important factor that strongly impacts the development of the gut microbiota in early life is the consumption of sow milk, which results in a milk-oriented microbiome (Frese *et al.*, 2015). Host genotype and other factors, such as environmental factors, also impact microbiota composition, although the underlying mechanisms for selection of specific microorganisms are currently poorly understood. A new phase of microbiota development starts when the pigs are reared on solid feed, which occurs after weaning. The transition to solid feed leads to an enrichment of functional pathways that are involved in plant glycan metabolism (Frese *et al.*, 2015), which relates to the plant-derived ingredients that are present in weaner diets. In addition to the changes in gut microbiota development, the sudden shift to solid feed can result in low or erratic feed intake, which is considered a risk factor for disease. Additional risk factors for disease include high dietary protein content, low levels of hygiene, low ambient air quality or low ambient air temperature (Lallès *et al.*, 2004). Just after weaning, the rapid changes in microbiota composition and activity result in a higher incidence of GI complications. These complications include a decrease in villus height (Hampson, 1986), villus atrophy (Al Masri *et al.*, 2015), reduced activity of intestinal digestive enzymes, disturbed intestinal permeability and the colonization of enteric pathogens such as *Escherichia coli* and rotaviruses (Lallès *et al.*, 2004).

Many pathogens found in pig herds are of viral origin, and vaccines are being developed to fight these viral diseases, such as influenza (Romagosa *et al.*, 2011), African Swine Fever (ASF) (Argilaguet *et al.*, 2012), Porcine Reproductive Respiratory Syndrome (PRRS) (Meng, 2000) and Porcine Circovirus type 2 (Rose *et al.*, 2016). Although comparatively less contagious than most viral diseases, bacterial-borne diseases, such as enterotoxigenic *Escherichia coli* (ETEC), *Streptococcus suis*, *Salmonella enterica* and *Clostridioides difficile* are also of economic importance because they can lead to pig mortality. Notably, within the EU approximately 17% of liveborn piglets are lost and of these losses, a substantial proportion can be associated with mucosal infections (Lallès *et al.*, 2007). The transition period of weaning is associated with GI tract infections, most often with colibacillosis diarrhoea (Gresse *et al.*, 2017). As advised by Luppi in 2017, when dealing with colibacillosis, it is important to diagnose which ETEC type is responsible and to test for antimicrobial sensitivity, because antimicrobial sensitivity is highly variable among *E. coli* isolates (Luppi, 2017). Enteric microbial infections can, although with clear drawbacks, typically be prevented and treated using antibiotics and/or antimicrobial supplements such as zinc oxide (ZnO) and copper sulphate. One of the drawbacks of the use of both antibiotics and antimicrobial supplements is the selection for antimicrobial resistant microbes (Moreno, 2014).

Globally, the rising rate of antimicrobial resistance, caused by widespread use of antibiotics, is a significant concern. Antibiotics used in livestock production are closely related to those used for humans, and cross-species transmission of resistant bacteria or

resistance-conferring genetic elements from animals to humans can occur (Tang *et al.*, 2017). Furthermore, the use of zinc and copper in pig feed has been shown to result in the presence of multi-drug resistant *Salmonella* (Medardus *et al.*, 2014), methicillin-resistant *Staphylococcus aureus* (Slifierz *et al.*, 2015) and multi-drug resistant *E. coli* (Bednorz *et al.*, 2013). Additionally, the use of zinc and copper at therapeutic dosage in pig feed is suspected to cause environmental pollution (Jondreville *et al.*, 2003). Frequent use of antibiotics may result in the spread of multi-resistant bacteria (Barton, 2014; Landers *et al.*, 2012), and concerns regarding public health have resulted in legislation reducing the use of antibiotics in livestock. To this end, the EU has banned the use of antimicrobial growth promoters (AGPs) in 2006 and has more recently decided to limit the use of ZnO to a maximum of 150 mg/kg in feed (Starke *et al.*, 2014). However, according to several studies, AGPs are still used in a prophylactic manner. In addition, ZnO is also used at therapeutic levels, which often exceed 150 mg/kg (Barton, 2014; Moreno, 2014; Rhouma *et al.*, 2016). Currently, the majority of antibiotics are still used in the livestock sector (Landers *et al.*, 2012), which emphasizes the need to develop non-antibiotic alternatives to prevent and control GI tract infections (Gresse *et al.*, 2017). There exists an extensive body of literature on non-microbial and microbial dietary interventions that have been tested in *in vivo* trials to assess their impact on the porcine GI tract microbiota. Of the dietary interventions tested, a number were shown to have a beneficial effect and as such could possibly be further developed and used as nonantibiotic alternatives.

This chapter will provide an overview of both the development of the microbiota in the GI tract of piglets and the effectors involved. It will also describe findings regarding the interaction between the microbiota and the host, the relation of microbiota composition to disease (predisposition) and of microbial and non-microbial feed additives that are used to ameliorate gut-related issues in early life.

7.2 Effectors in early-life microbiota programming

The stomach functions as a pre-digestive organ that prepares the ingested feed for digestion at a low pH and that mixes solids with liquids. In the small intestine nutrients are hydrolysed by digestive enzymes and are transported across the epithelium. The large intestine acts as a fermentation chamber where mostly fibres and other indigestible feed components are broken down by anaerobic bacteria into components that can be absorbed and used by the host, such as short chain fatty acids (SCFAs). The SCFAs are important because they are used as an energy source by the host. In addition to allowing a proper fermenting capacity with the corresponding release of breakdown products in the GI tract, the gut microbiota is important for the development of gut physiology, immunity, and general well-being of the piglet (Guevarra *et al.*, 2019). Most studies have demonstrated that *Firmicutes* and *Bacteroidetes* are the two main phyla with the highest relative abundance along the entire GI tract and in the faeces of piglets (Gresse *et al.*, 2017; Kim *et al.*, 2011; Looft *et al.*, 2014). This is in contrast to some studies that showed exceptions to this finding, with one study that indicated *Firmicutes* and *Proteobacteria* to be the main phyla in both the jejunum and the ileum (Yang *et al.*, 2016). Additionally, most studies have found an increasing trend of microbiota diversity as the piglets age

(Chen *et al.*, 2017; Wang *et al.*, 2019a). At birth, the piglet's gut is exposed to microbes for the first time and these microbes originate from the sow's vagina during the piglet's passage through the birth canal. Subsequently, piglets are exposed to microbes in the housing environment, including microbes from the sow's faeces and microbes from the sow's skin during lactating. As few niches of the gut ecosystem are occupied in the first days after birth, many of these early microbial exposures lead to successful colonization. In general, exposure to microbes in the early stages of postnatal life is not considered detrimental, since the development of the gut microbiota requires continuous microbial exposure (Schmidt *et al.*, 2011).

As mentioned in the introduction, the GI tract is a largely oxic environment after birth. This results in the acquisition of oxygen tolerant bacteria during the first 24 hours, such as lactic acid bacteria, enterobacteria and streptococci (Lallès *et al.*, 2007). Other bacteria such as *Bacteroides*, *Lactobacillus*, *Bifidobacterium* and *Clostridium* colonize shortly after the first colonizers, namely, within one to five days after birth (Petri *et al.*, 2010). In one study a stable microbiota composition was observed from the first day after birth and remained for the first three weeks. It mainly consisted of *Enterobacteriaceae*, *Lachnospiraceae*, *Bacteroidaceae*, *Clostridiaceae* and *Lactobacillaceae* (Frese *et al.*, 2015). This is in contrast to what happens in human neonates, where early colonizers such as *Lactobacillus* and proteobacteria are supplanted during the first weeks of postnatal life by bacterial taxa that can feed on components of mother milk, such as bifidobacteria and *Bacteroides* (Dominguez-Bello *et al.*, 2010; Orrhage and Nord, 1999). Several studies have shown the relevance of early-life gut microbiota development for gut health later on. A study by Dou *et al.* (2017) showed that a higher relative abundance of particular microbial groups during the nursing stage profoundly affects the emergence of post-weaning diarrhoea. Pigs that did not develop diarrhoea during the post-weaning period displayed a higher relative abundance of *Prevotellaceae*, *Lachnospiraceae*, *Ruminococcaceae* and *Lactobacillaceae* at day 7 of age compared to diarrhoeic pigs. In another study it was shown that administering multiple species of microbes to sows during gestation and lactation influenced the composition of the microbiota and the concentration of SCFAs in the faeces of piglets during the post-weaning phase (Mori *et al.*, 2011). These studies illustrate the potential for applying microbial interventions in the first weeks of postnatal life.

Host genotype was also shown to play a crucial role in the development of the microbiota along the GI tract of piglets. In a study by Xiao *et al.* (2018a), the microbiota composition along the GI tract of piglets from two separate breeds (Jinhua vs Landrace) was investigated using 16S rRNA gene sequencing. The microbial diversity in the duodenum, jejunum and caecum was found to differ between the two breeds, whereas the ileal and colonic microbial diversity was similar. Furthermore, several bacterial groups were different in terms of relative abundance along the entire GI tract between the two breeds, with jejunal and ileal microbiota showing the greatest contrast. In addition to genotypic and microbiota differences between breeds, differences within pig breeds also exist, albeit smaller. Pig breeders typically select for general traits such as growth performance and overall health, but many breeders also take into account specific genotypic traits such as the absence of particular receptors along the gut lining that provide elevated colonization resistance

against particular pathogens. One example is the breeding of F4 receptor-negative (F4R⁻) pigs, which prevents the adhesion and subsequent infection by the diarrhoea causing pathogen ETEC harbouring F4 (K88)⁺ fimbriae (Zhou *et al.*, 2015). Although this Mucin 4 gene polymorphism has been shown to affect susceptibility to ETEC, it was also shown to have an effect on bacterial diversity in the jejunum, which might indicate that the interplay between the host and the microbiota is affected by the presence of this receptor (Messori *et al.*, 2013). In addition to the Mucin 4 gene, the Mucin 13 gene is also of relevance because one of its two transcripts was reported to be associated with efficient binding by strains of *E. coli* that have F4 fimbriae (Ren *et al.*, 2012).

Several effectors (i.e. diet, age) have a strong impact on composition of the microbiota along the GI tract. In order to find out which effectors are most important, the relative contribution of effectors can be compared. For example, in one study it was shown that age, introduction of solid feed and weaning were more important determinants of gut bacterial succession in piglets than breed and nursing mother (Bian *et al.*, 2016). Nevertheless, piglet breed and nursing sow breed did lead to increasing differentiation of the piglet's faecal bacterial community, with specific bacterial taxa being associated with either piglet breed or sow breed, the latter most likely due to differences in milk composition (Bian *et al.*, 2016). The introduction of creep feed is similarly expected to have a measurable impact on composition of the microbiota although, to the best of our knowledge, a trial specifically dedicated to exploring this has yet to be published. In addition to the more obvious factors affecting microbiota composition that were previously mentioned, factors like sanitary conditions (Montagne *et al.*, 2010), dam parity (Carney-Hinkle *et al.*, 2013) and housing (Kubasova *et al.*, 2017) also have a measurable impact on microbiota composition and as such should be taken into consideration.

7.3 Effectors of post-weaning development of microbiota

Weaning is a critical event in a pig's life. When a piglet switches from sow milk to solid feed as a source of nutrients, it is a sudden and stressful event which strongly impacts piglet health (Campbell *et al.*, 2013). Indeed, the piglet's health is affected by the change in diet and also by the accompanying social and environmental stresses. These stresses include the separation from the mother, handling, transport, changing physical environments and mixing of litters (Campbell *et al.*, 2013). In conventional pig husbandry practices, piglets are weaned at approximately 3-4 weeks of age, while natural weaning would occur much later at around 17 weeks of age (Jensen, 1986). This difference between conventional weaning and natural weaning is mostly due to economic reasons but also exists to prevent damaging of sow teats, which occurs more frequently as piglets become older. Weaning can lead to anorexia, which contributes to local inflammation in the piglet's small intestine (McCracken *et al.*, 1999). Furthermore, weaning is frequently associated with severe enteric infections and a high prevalence of enteric infections leads to overuse of antibiotics, which raises economic and public health concerns (Gresse *et al.*, 2017). In addition to these concerns, antibiotics have been shown to have long-lasting effects on microbiota composition and host gene expression in the piglet, even when administered only once in the first week after birth (Schokker *et al.*, 2015). Similarly,

administration of antibiotics to sows around parturition was shown to result in great differences in gut physiology of the offspring both during early neonatal life and at six months of age, and this especially in response to a dietary challenge with a high fat diet (Arnal *et al.*, 2014, 2015). Antibiotic use can be reduced if the frequency of enteric infections is reduced. To prevent enteric infections at weaning, it is important that the intestinal microbiota develops into a complex and stable community that generates a tight 'colonisation resistance' or 'competitive exclusion' against pathogens (Lallès *et al.*, 2007).

At weaning, the switch from highly digestible sow milk to less-digestible solid feed results in a shift from milk-derived glycan metabolism to plant-derived glycan metabolism along the GI tract (Frese *et al.*, 2015). The microbiota composition changes dramatically (Konstantinov *et al.*, 2006) with, for example, an increase in the relative abundance of obligate anaerobes (Inoue *et al.*, 2005). This shift of the gut microbiota is considered to be a main factor leading to post-weaning diarrhoea. As reviewed by Gresse *et al.* (2017), most studies conducted on the weaning transition have reported a decrease in bacteria of the *Lactobacillus* group accompanied by a loss of microbial diversity. In addition, an increase was reported for *Clostridium* spp., *Prevotella* spp. and *Proteobacteriaceae* (Gresse *et al.*, 2017), while several studies reported a decrease of *E. coli* after weaning (Mach *et al.*, 2015; Pajarillo *et al.*, 2014; Wang *et al.*, 2019a). The decrease in gut microbial diversity might be the leading cause for post-weaning diarrhoea, but the weaning transition is also associated with increased gut permeability and intestinal inflammation (Brown *et al.*, 2006). Although the weaning transition entails a highly dynamic period in terms of microbiota succession that lasts approximately 1-2 weeks, changes in microbiota composition and further maturation still occur after this period. For example, it was found that the most predominant genus *Prevotella* represented up to 30% of all classifiable bacteria when pigs were 10 weeks of age, while this percentage decreased to 3.5-4.0% when pigs were 22 weeks of age. With this decrease in *Prevotella*, an increase in *Anaerobacter* was observed. In the same time frame, an increase in the phylum *Firmicutes* was observed, whereas the proportion of bacteria in the phylum *Bacteroidetes* decreased (Kim *et al.*, 2011). Most studies to date have focused on the period up to and during the weaning transition but only few studies have addressed later periods. As more longitudinal studies are carried out to investigate the dynamics of the GI tract microbiota of pigs well after weaning, hopefully a more complete picture can be obtained of the maturation of pig gut microbiota during the later stages of life.

7.4 Interaction of microbiota with the host

Commensal gut microorganisms are important for the fermentation of dietary components and for competitively excluding colonization and growth of pathogens. However, these microorganisms may also interact with the porcine host cells and the immune system. Of the porcine host cells, the first that the gut microorganisms come into contact with are the epithelial cells. Commensal gut microorganisms affect the intestinal epithelial barrier (i.e. activation of regulatory pathways) and, in turn, the intestinal epithelial barrier develops tolerance towards their presence (Sharma *et al.*, 2010). In addition, microbial colonization has a marked effect on the formation of lymphoid tissues and corresponding immune

system development. Although this finding does not originate from a porcine study, the effect of microbial colonization was reported when using an alternative mammalian model, namely the comparison between germ-free and colonized mice (Hooper *et al.*, 2012). With respect to porcine research, antibiotic treatment in the first week after birth was shown to have long-lasting effects on host intestinal gene expression (Schokker *et al.*, 2015). The presence of several taxonomical groups of bacteria, including *Turicibacter*, *Clostridiaceae*, *Streptococcaceae* and *Lactobacillaceae*, was positively correlated with body weight, which may partly be explained by interactions with the host immune system (Wang *et al.*, 2019a). Another bacterium that was found to be positively correlated with pig body weight is *Lactobacillus mucosae*, which is known for its mucus-binding activity and for its ability to improve barrier function of the epithelium (Wang *et al.*, 2019a). The possibility for specific microbiota to adhere to the gut lining of the host strongly dictates the interactions that take place. In this respect the genome of the host is an important player as it dictates the presence or absence of fimbriae-specific receptors on the small intestinal microvilli to which specific microorganisms can adhere. Of these fimbriae-specific receptors, the F4 and F18 fimbriae-specific receptors have been studied most intensively (Nagy and Fekete, 2005) due to their relevance in post-weaning diarrhoea. The F4 receptors are expressed in intestinal tissue from birth, whereas F18 receptors are only fully expressed after three weeks of age (Fairbrother *et al.*, 2005). Many pathogenic strains are adapted to bind to these receptors. For example, most ETEC strains that cause neonatal colibacillosis carry the F4 (K88), F5 (K99), F6 (987P) or F41 fimbriae, while most ETEC strains that cause post-weaning diarrhoea carry the F4 and F18 fimbriae, with which they bind to receptors that are present on the porcine host's enterocytes (Luppi, 2017). Furthermore, F4 ETEC is able to colonize along the entire length of the jejunum and ileum, while F5, F6 and F41 ETEC mostly colonize the posterior jejunum and ileum (Luppi, 2017). Research in human subjects has elucidated the chain of events that lead to diarrhoea caused by ETEC strains. The ETEC strains produce enterotoxins that increase cellular electrolyte and fluid secretion, leading to hyper-secretory diarrhoea (Croxen and Finlay, 2010). In response to exposure to F4 ETEC bacteria, the piglet's gut epithelium reacts by increasing the expression of specific genes, such as those encoding TLR4 and IL-8 in the jejunum and porcine β -defensin 2 in the ileum (Li *et al.*, 2012c). To summarize, there is an ongoing interaction between the intestinal gut microbiota and the porcine host, and by adjusting the expression of genes along the gut lining the porcine host is able to actively respond to the presence of pathogens.

7.5 Relation between microbiota status and disease susceptibility

The composition of the microbiota in animals is of importance as it determines to a certain extent whether these animals are susceptible to enteric infections. In one study it was shown that pigs that became low-*Salmonella*-shedding after a challenge with *S. enterica* serovar Typhimurium, had a higher relative abundance of the *Ruminococcaceae* family before challenge than the high-shedding pigs. In addition, the high shedding pigs showed a decrease in relative abundance of *Prevotella* 2 days post-inoculation, and increases in relative abundance of various other genera, such as *Catenibacterium* and *Xylanibacter* (Bearson *et al.*, 2013). Similarly, the inclusion of a *Saccharomyces*

cerevisiae fermentation product in the diet just after weaning altered the composition of the GI microbiota, which resulted in higher relative abundances of *Bacteroidetes* and *Lactobacillus* after infection with *Salmonella* (Price *et al.*, 2010). In a more recent study, it was also found that colonization resistance to *Salmonella* was influenced by the intestinal microbiota. Members of *Clostridia* and cellulolytic microorganisms, such as *Ruminococcus* and *Prevotella*, were positively linked to colonization resistance to *Salmonella* while microorganisms associated with suckling, such as *Lactobacillus* and *Oscillospira*, were present at higher relative abundance in piglets that became infected (Argüello *et al.*, 2019). These last authors suggested that a lack of microbiome maturation increased susceptibility to infection. In summary, experiments that included a *Salmonella* challenge showed that the predisposition of the microbiota is of relevance for the subsequent succession of disease in piglets. Although these experiments do provide clues as to which taxonomic groups might be helpful to increase colonization resistance against particular pathogens, more research is required to better understand these interactions and to decipher through which means and when to best steer GI microbiota development towards desired endpoints.

7.6 Microbiota-inspired strategies to mitigate gut related issues

As previously discussed, the composition and functionality of the microbiota plays a crucial role for the health of the porcine host and is influenced by multiple effectors including colonization during and after delivery, diet and host genotype. Furthermore, feed efficiency and other growth traits are linked to microbiota composition or with specific members of the gut microbiota (McCormack *et al.*, 2017; Ramayo-Caldas *et al.*, 2016; Xiao *et al.*, 2018b). In addition to growth traits, gut related health issues such as pathogen infection, reduced gut barrier function and diarrhoea are linked to composition of the gut microbiota. Strategies that are aimed at reducing the incidence of microbiota-related complications involve the timely presentation of creep feed, vaccination against enteric pathogens, limitation of protein content in feed, inclusion of feed ingredients that can reduce gastric pH, and exclusion of feed ingredients that can increase gastric pH. To date, several *in vivo* studies have been carried out to look at the effect of creep feed intake on post-weaning feed intake (Bruininx *et al.*, 2002) and on post-weaning occurrence of colibacillosis (Carstensen *et al.*, 2005). It was shown that consumption of an adequate (not too much and not too little) amount of creep feed during the nursing period stimulates early post-weaning feed intake, while occurrence of diarrhoea and faecal shedding of haemolytic *E. coli* were not conclusively associated with creep feeding.

Although vaccines are generally used to raise immunity against specific pathogens and are generally not designed to have an impact on the overall composition of the microbial community, the use of vaccines aimed at enteric pathogens can be identified as a microbiota-inspired strategy to mitigate gut related issues. Considering that several vaccines against enteric pathogens are widely used today and have proven their efficacy, the use of vaccinations is mentioned here among the possible strategies used by livestock producers to alter microbiota composition. Several vaccines were developed to protect piglets against pathogenic *E. coli* strains, such as strains that produce the Shiga-like toxin

II variant (SLT-IIe) (Bosworth *et al.*, 1996). One such vaccine that was commercialized is Coliprotect. This vaccine provides sufficient protection to piglets against a challenge with ETEC F4 to significantly reduce the duration and severity of diarrhoea (Melkebeek *et al.*, 2013). Vaccination of piglets against *Salmonella* has resulted in mixed results, likely due to the complex infection strategy of *Salmonella*, with several studies showing reduced presence/shedding of *Salmonella* in the mesenteric lymph nodes (MLNs) or faeces (Denagamage *et al.*, 2007). More recently, a number of vaccination trials showed promising results (Wales and Davies, 2017), including the trial by Roesler and co-authors, where significant protection against clinical signs, shedding and tissue invasion was observed (Roesler *et al.*, 2010). As has been reviewed, more recent approaches using mucosal presentation of antigens have been relatively successful, most likely due to the enhancement of cell-mediated immunity and mucosal immunity (Wales and Davies, 2017).

A review by Rist and co-authors reported that avoiding excessive amounts of protein that reach the lower GI tract may reduce the incidence of post-weaning diarrhoea and inhibit proliferation of pathogenic bacteria (Rist *et al.*, 2013). In addition, a moderate reduction of dietary protein level may reduce the formation of detrimental fermentation products, such as ammonia, hydrogen sulphide and biogenic amines. Moreover, there are indications that the inclusion of fermentable carbohydrates could be promising to reduce fermentation of detrimental proteins and proliferation of potential pathogenic proteolytic bacteria (Rist *et al.*, 2013). In a pig trial focusing on the efficacy of a vaccine, the effect of dietary protein content was tested in parallel. Subclinical oedema disease developed in the pigs fed a low-protein diet, while pigs fed a high protein diet developed clinical oedema disease and death (Bosworth *et al.*, 1996). Reduction of post-weaning diarrhoea can also likely be achieved by limiting dietary protein content for a short period after weaning. However, caution should be taken to provide sufficient protein to match the piglet's requirements, as otherwise growth performance might be compromised (Halas *et al.*, 2007). Furthermore, it has been shown that a greater dietary crude protein (CP) content (i.e. 230 vs 130 g CP/kg) increased coliforms in faeces and decreased the lactobacilli to coliform ratio in the proximal colon (Wellock *et al.*, 2006). Although increasing crude protein content in feed led to an increase in faecal fluidity, it contrastingly also led to improvements in average daily gain. Consequently, with the inclusion of crude protein, there seems to be a trade-off between maximizing performance and minimizing risks of post-weaning diarrhoea. Interestingly, it was shown that feeding a low protein diet (173 g CP/kg) supplemented with amino acids to conform to an ideal amino acid pattern can reduce post-weaning diarrhoea without compromising production (Heo *et al.*, 2008). Taken together, these results indicate that limiting dietary protein content is a viable option to lower the abundance of coliforms and to reduce gut related issues in piglets, especially when a reduction in the use of AGPs is desired.

The acidity of the stomach is the first line of defence against incoming pathogens and as such is important to take into account in the prevention of gut related issues. The incidence of pathogen survival and their transit to the small intestine are increased by the variability (between 2.2 and 4.2) of the pH in the piglet's stomach (Kim *et al.*, 2012). Overeating in the early weaning phase causes a greater risk for elevated gastric

pH which may result in a higher incidence of pathogen survival (Halas *et al.*, 2007). The use of feed ingredients that reduce gastric pH (e.g. organic acids), or the exclusion of feed ingredients that increase gastric pH (e.g. calcium carbonate) seem to be viable options to prevent gut-related problems. Of note, due to the buffering capacity of some amino acids, such as lysine, arginine and histidine, protein content is believed to have an influence on gastric pH as well (Partanen and Mroz, 1999). In addition to the strategies mentioned, the incorporation of feed additives is a means of improving gut health and can be used to alter gut microbiota composition. Table 7.1 summarizes results from *in vivo* studies using currently fed additives and investigating their effects on the composition of the gut microbiota. One of the most widely used class of feed additives are the AGPs, which have been used since the 1950s. Although the modes of action of AGPs are still poorly understood, they have provided benefits such as increases in growth performance and reduction of disease incidence. Researchers have found that AGPs have an effect on bacterial load and composition and that they also interact with the immune system (Brown *et al.*, 2017).

Promising alternatives to AGPs include feed additives such as prebiotics, probiotics, organic acids, minerals and essential oils (Pluske *et al.*, 2018). Prebiotics such as resistant starch, pectin, β -glucans and oligosaccharides are used to enhance bacterial fermentation of dietary fibres in the hindgut. Intestinal physiology and microbial composition, hence microbial fermentation, were shown to be influenced by these ingredients (Hopwood and Hampson, 2003). Prebiotics have therefore provided livestock producers a 'clean-label' feeding option that may be used to mitigate the negative effects of morbidity on growth and performance (Broadway *et al.*, 2015).

7.7 The effect of non-microbial feed interventions on microbiota composition

A number of fibrous feed components (e.g. beta-glucans, cello-oligosaccharides and arabinoxylans) have been tested for their effects on the composition of the GI tract microbiota (Table 7.1). Generally, these fibrous components are not digested by the host's enzymes but are instead digested by the fermenting capacity of fibre-degrading bacteria in the hindgut. This microbial fermenting activity results in the production of metabolites such as SCFAs, of which butyrate is an important source of energy for enterocytes. In addition, some polysaccharides, such as yeast-derived polysaccharides, which are mostly comprised of α -D-glucans and β -D-glucans (Kogan and Kocher, 2007), can also interact directly with host immune cells and are able to bind bacteria while blocking fimbriae of pathogenic bacteria and preventing their attachment to the mucous epithelium in the GI tract (Kogan and Kocher, 2007; Ruiz-Herrera, 2016). Short- and medium-chain organic acids such as lactic, citric, butyric, propionic and fumaric acids are used in piglet feed because of their bactericidal effect during feed storage, their effect at lowering gastric pH and because they help to improve growth performance (Gresse *et al.*, 2017; Suiryanrayna and Ramana, 2015). Proposed mechanisms by which growth performance is improved include the impact of these organic acids on digestive processes, their utilization as energy source and their effect on the composition of the intestinal microbiota. The use of

Table 7.1. Influence of non-microbial feed additives on porcine microbiota composition. Of note: this overview is not a result of a systematic approach.

Treatment	Effects	Location	Reference
Antibiotics			
Tylosin, post-weaning	> lactobacilli (only bacterial group that was tested using qPCR)	ileum	Collier <i>et al.</i> (2003)
Chlortetracycline, sulfamethazine and penicillin, post-weaning	> proteobacteria, <i>Succinivobrio</i> and <i>Ruminococcus</i> , < <i>Bacteroidetes</i>	ileum	Collier <i>et al.</i> (2003)
Chlortetracycline, post-weaning	< <i>Lactobacillus johnsonii</i> and <i>Turicibacter</i> , > <i>Lactobacillus amylovorus</i>	ileum	Rettedal <i>et al.</i> (2009)
Tulathromycin, pre-weaning	< lactobacilli (trend)	jejunum	Schokker <i>et al.</i> (2014)
Lincomycin, post-weaning	> <i>Lactobacillus</i> (only bacterial group that was tested using qPCR)	ileum	Gong <i>et al.</i> (2008)
Heavy metals			
Zinc oxide, post-weaning	< <i>Enterobacteriaceae</i> and <i>Lactobacillaceae</i>	ileum	Starke <i>et al.</i> (2014)
Zinc oxide, post-weaning	< non-specific post-weaning diarrhoea	faeces	Poulsen (1995)
Zinc oxide, post-weaning	> <i>Enterobacteriales</i>	ileum, colon	Yu <i>et al.</i> (2017)
Zinc oxide, post-weaning	> <i>Weissella</i> spp., <i>Leuconostoc</i> spp., <i>Streptococcus</i> spp., < <i>Sarcina</i> spp., <i>Neisseria</i> spp., > gram-negative facultative anaerobic genera	ileum	Vahjen <i>et al.</i> (2010)
Zinc oxide, post-weaning	< <i>Bifidobacterium</i> spp.	faeces	Mukhopadhyaya <i>et al.</i> (2019)
Zinc oxide, post-weaning	< lactobacilli, > enterococci	multiple segments GI tract	Højberg <i>et al.</i> (2005)
Copper sulphate, post-weaning	< coliforms	colon	Højberg <i>et al.</i> (2005)
Organic acids			
Mix of organic acids: formic, benzoic, sorbic acid and butyrate, post-weaning	< coliforms, enterococci, lactic acid producing bacteria	jejunum, colon, rectum	Øverland <i>et al.</i> (2008)
Mix of organic acids, post-weaning	> <i>Lactobacillus</i> (only bacterial group that was tested using qPCR)	ileum	Gong <i>et al.</i> (2008)
Prebiotics			
Resistant starch, post-weaning	> <i>Faecalibacterium prausnitzii</i> , < <i>Escherichia coli</i> , <i>Pseudomonas</i>	colon	Haenen <i>et al.</i> (2013)
Inulin, pre-weaning	> <i>Lactobacillus</i> and <i>Bifidobacterium</i>	caecum	Tako <i>et al.</i> (2008)
Inulin, post-weaning	> Pigs harbouring bifidobacteria	colon	Loh <i>et al.</i> (2006)
Lactose, post-weaning	> <i>Bifidobacterium</i>	faeces	Pierce <i>et al.</i> (2006)
Lactose, post-weaning	< <i>E. coli</i>	faeces	O'Doherty <i>et al.</i> (2010)
Lactulose, post-weaning	> lactobacilli	colon	Guerra-Ordaz <i>et al.</i> (2014)
Lactulose, post-weaning	< proteobacteria, > <i>Clostridium</i>	faeces	Chae <i>et al.</i> (2016)
FOS, post-weaning	> <i>Bifidobacterium</i> , < <i>E. coli</i>	colon	Gebbink <i>et al.</i> (1999)
GOS, post-weaning	> <i>Bifidobacterium</i>	colon	Tzortzis <i>et al.</i> (2005)
Oat-derived β -glucans, post-weaning	> lactobacilli and bifidobacteria	colon	Metzler-Zebeli <i>et al.</i> (2011)
Yeast-derived β -glucans, post-weaning	< <i>Enterobacteriaceae</i>	colon	Sweeney <i>et al.</i> (2012)

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Table 7.1. Continued.

Treatment	Effects	Location	Reference
Prebiotics (continued)			
Arabinoxylans, post-weaning	> <i>F. prausnitzii</i> , <i>Roseburia intestinalis</i> , <i>Blautia coccooides</i> , <i>Eubacterium rectale</i> , <i>Bifidobacterium</i> spp., <i>Lactobacillus</i> spp.	faeces	Nielsen <i>et al.</i> (2014)
Cello-oligosaccharides, post-weaning	> lactobacilli, < <i>Streptococcus suis</i> , < <i>E. coli</i>	jejunum, colon	Jiao <i>et al.</i> (2015)
Pectin, post-weaning	< <i>Lactobacillus</i> , > <i>Prevotella</i>	colon	Tian <i>et al.</i> (2017)
Mannanoligosaccharides, post-weaning	< <i>Enterobacteriaceae</i>	jejunum	Castillo <i>et al.</i> (2008)
Seaweed extract, post-weaning	< <i>E. coli</i>	faeces	O'Doherty <i>et al.</i> (2010)
Chicory forage and root, post-weaning	> lactic acid bacteria	ileum	Liu <i>et al.</i> (2012)
Chicory forage and root, post-weaning	> butyrate producing bacteria, > <i>Megasphaera elsdenii</i>	colon	Liu <i>et al.</i> (2012)
Essential oils			
Carvacrol-thymol, post-weaning	> <i>Lactobacillus</i> , < <i>Enterococcus</i>	jejunum	Wei <i>et al.</i> (2017)
Cinnamaldehyde-thymol, post-weaning	< <i>E. coli</i> , < total anaerobes	rectum	Zeng <i>et al.</i> (2015)
Cinnamon-thyme-oregano extract, post-weaning	< coliforms	faeces	Namkung <i>et al.</i> (2004)
Cinnamaldehyde-thymol, post-weaning	< <i>E. coli</i> , > ratio lactobacilli/ <i>E. coli</i>	caecum, colon and rectum	Li <i>et al.</i> (2012a)
Cinnamaldehyde-thymol, post-weaning	< <i>E. coli</i> (based on counts of <i>Lactobacillus</i> and <i>E. coli</i> counts)	faeces	Li <i>et al.</i> (2012b)
Herbal extract, post-weaning	> <i>Lactobacillus</i> (only bacterial group that was tested using qPCR)	ileum	Gong <i>et al.</i> (2008)
Fungal compounds			
Pulverized oyster mushroom (<i>Pleurotus ostreatus</i>), post-weaning	< Incidence of diarrhoea, < <i>Ruminococcaceae</i> (all doses), > <i>Veillonellaceae</i> (at 10 g/kg dose)	faeces	Adams <i>et al.</i> (2019)
Freeze-dried white button mushroom (<i>Agaricus bisporus</i>), post-weaning	> <i>Lachnospiraceae</i> , > <i>Ruminococcaceae</i> , < <i>Bifidobacteriaceae</i>	faeces, proximal colon	Solano-Aguilar <i>et al.</i> (2018)
Lentinan (Mushroom polysaccharide from <i>Lentinus edodes</i>), post-weaning	< <i>Firmicutes</i> , > <i>Bacteroidetes</i> , > <i>Faecalibacterium</i> , > <i>Prevotella</i>	caecum	Wang <i>et al.</i> (2019b)

organic acids in newly weaned piglets has been mostly related to a decrease in coliforms and an increase in *Lactobacillus* along the GI tract (Gong *et al.*, 2008; Gresse *et al.*, 2017), although some studies have shown a decrease in *Lactobacillus* (Gedek *et al.*, 1992; Maribo *et al.*, 2000; Øverland *et al.*, 2008). Even though it presents some drawbacks, ZnO was proposed as one of the most effective feed additives to replace antibiotics and is already widely commercialized in several countries. High levels of ZnO were shown to have antimicrobial properties and are currently used to fight against post-weaning infections. The most severe effects of high dietary zinc were observed one week after weaning in the stomach and small intestine, with pronounced reductions of *Enterobacteriaceae* as well as

lactobacilli (Starke *et al.*, 2014). Others have also found a reduction in *Lactobacillus* spp. after treatment with ZnO (Broom *et al.*, 2006; Højberg *et al.*, 2005; Vahjen *et al.*, 2011), although the reduction of *Enterobacteriaceae* was not found in all studies (Højberg *et al.*, 2005; Vahjen *et al.*, 2011).

Another group of compounds that is used as a dietary supplement for pigs belongs to the phytochemical feed additives. This group comprises additives such as herbs, spices, essential oils and oleoresins. Although results are variable, their inclusion can result in improved feed intake by enhancing palatability, and can also increase storage preservation of the feed. Additional beneficial effects include improved digestive secretions, improved antioxidative status and antimicrobial effects (Jacela *et al.*, 2010). With respect to microbiota composition, *in vivo* studies that have included dietary essential oils generally resulted in a reduction in the amount of *E. coli* and/or other Gram-negative bacteria (Table 7.1). Another group of feed additives that has recently received attention involves the fungal kingdom. To date, there are two studies in which dehydrated and ground mushroom fruiting bodies (white button mushroom/oyster mushroom) have been added to feed and one study in which a particular polysaccharide (lentinan) from the Shiitake mushroom was used in feed for swine. Although results are variable between mushroom species, these fungal feed additives positively affected the composition of the piglet GI tract microbiota after weaning, with increases in fibre-degrading and SCFA-producing bacterial groups. In addition, a reduction in the incidence of diarrhoea with the administration of pulverized oyster mushroom powder was observed (see summary of findings in Table 7.1).

7.8 The effect of microbial feed interventions on microbiota composition

Apart from the use of antibiotics or other non-microbial feed components, the GI tract microbial composition can also be altered with microbial interventions. These can exist as natural or synthetic mixed culture inocula, fermented products, probiotics or synbiotics. Table 7.2 provides an overview of studies in which the effect of microbial interventions on gut microbiota composition was tested. Studies that were conducted before the year 2000 were not included in this table because a summary was made by Shim and colleagues in 2005 (Shim *et al.*, 2005b), showing that probiotics are beneficial to a certain degree and are able to influence some beneficial bacteria in the GI tract of young piglets. The mechanisms of action of microbial interventions include competition with pathogens for mucosal binding sites along the GI tract, competition for nutrients in the lumen, production of antimicrobial compounds such as microcins, and production of organic acids that inhibit pathogen growth (Vondruskova *et al.*, 2010). Additionally, some probiotics have strong adherence capacities to bind to intestinal epithelial cells or to the mucus layer (Bezkorovainy, 2001), and several probiotics are thought to be capable of enhancing mucosal barrier integrity (Roselli *et al.*, 2017). Strongly adhering probiotics are expected to interfere with the adhesion of pathogenic bacteria and the adherence is also associated with stimulatory effects on the immune system (Isolauri *et al.*, 2001). Another effect that can occur through the use of microbial interventions is the modulation of the composition and activity of the resident microbiota (Gresse *et al.*,

Table 7.2. Influence of probiotics on porcine microbiota composition. Of note: this overview is not a result of a systematic approach.

Treatment	Effects	Location	Reference
Probiotics			
Complex lactobacilli preparation, post-weaning	> <i>Escherichia coli</i> , < lactobacilli	most sections of GI tract	Huang <i>et al.</i> (2004)
<i>Bifidobacterium longum</i> , post-weaning	< anaerobes and clostridia, > bifidobacteria	faeces	Estrada <i>et al.</i> (2001)
<i>Lactobacillus sobrius</i> , post-weaning	< ETEC	ileum	Konstantinov <i>et al.</i> (2008)
<i>Enterococcus faecium</i> , post-weaning	> lactobacilli	ileum, caecum and faeces	Mallo <i>et al.</i> (2010)
<i>Bacillus subtilis</i> , post-weaning	< <i>Clostridium</i> spp. and coliforms	caecum	Lee <i>et al.</i> (2014)
<i>Lactobacillus plantarum</i> , post-weaning	> lactobacilli	colon	Guerra-Ordaz <i>et al.</i> (2014)
<i>Enterococcus faecalis</i> , post-weaning	> lactobacilli	faeces	Hu <i>et al.</i> (2015)
<i>Lactobacillus johnsonii</i> , post-weaning	> lactobacilli, < <i>E. coli</i>	faeces	Chiang <i>et al.</i> (2015)
<i>Lactobacillus mucosae</i> , post-weaning	> lactobacilli, < <i>E. coli</i>	faeces	Chiang <i>et al.</i> (2015)
<i>Lactobacillus acidophilus</i> , post-weaning	> lactic acid bacteria and bifidobacteria, < <i>E. coli</i> and clostridia	faeces	Dowarah <i>et al.</i> (2017)
<i>Pediococcus acidilactici</i> , post-weaning	> lactic acid bacteria and bifidobacteria, < <i>E. coli</i> and clostridia	faeces	Dowarah <i>et al.</i> (2017)
Multispecies probiotic mix, pre-weaning	< <i>Clostridium</i> spp.	distal colon	Barszcz <i>et al.</i> (2016)
Mix of <i>Bacillus licheniformis</i> and <i>Bacillus subtilis</i> followed by F4 ⁺ ETEC challenge, post-weaning	> <i>Clostridium</i> , <i>Lactobacillus</i> and <i>Turicibacter</i>	colon	Zhang <i>et al.</i> (2017)
<i>Lactobacillus rhamnosus</i> GG, post-weaning	no effect on counts of lactic acid bacteria, enterobacteria and yeasts	colon	Trevisi <i>et al.</i> (2011)
<i>E. coli</i> Nissle 1917, post-weaning	< diversity of <i>E. coli</i> strains	mucosal gut smears along GI tract	Šmajš <i>et al.</i> (2012)
Synbiotics			
Oligofructose with probiotic mixture in creep feed, pre-weaning	< coliforms, > bifidobacteria	ileum and colon	Shim <i>et al.</i> (2005a)
Raw potato starch with <i>E. coli</i> probiotics, pre-weaning	reduction of diarrhoea, increased microbial diversity	ileum and colon	Krause <i>et al.</i> (2010)
Lactulose with <i>Enterococcus faecium</i> , post-weaning	> <i>Lactobacillus</i> , < <i>Enterobacteriaceae</i>	faeces	Chae <i>et al.</i> (2016)
FOS and <i>Bifidobacterium thermophilum</i> , post-weaning	> <i>Bifidobacterium</i> in synbiotic group compared to probiotic alone	caecum and colon	Tanner <i>et al.</i> (2015)
Inulin with probiotic formulation (<i>E. faecium</i> , <i>L. salivarius</i> , <i>L. reuteri</i> , <i>B. thermophilum</i>), post-weaning	< <i>E. coli</i> , also changes in diversity and community profile	multiple locations along GI tract	Sattler <i>et al.</i> (2015)
Long-chain inulin combined with <i>Lactobacillus acidophilus</i> W37	> <i>Prevotellaceae</i> , < <i>Lactobacillaceae</i>	faeces	Lépine <i>et al.</i> (2019)

2017). Microorganisms that are commonly used as probiotics in livestock production systems are strains of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Bacillus*, *Pediococcus*, *Saccharomyces*, *Aspergillus* and *Escherichia coli*, and these can be administered via pelleted feed, as fermented feed, capsules, paste, powder and as granules (Ohashi and Ushida, 2009). Additionally, probiotics can be administered in liquid form, given that they are administered in an amount of fluid that will be ingested entirely and rapidly to avoid loss of viability or contamination by pathogenic bacteria (Tournut, 1989).

In recent years, on-farm fermentation of feed has become increasingly popular, as small-scale fermenting systems that can be used to perform controlled fermentations have become available. It is essential to perform the fermentation in a controlled manner because uncontrolled fermentation can lead to the production of acetic acid and deterioration of the feed conversion of weaning pigs (Van der Peet-Schwering *et al.*, 2004). Typically, an inoculum of a single (or multiple) *Lactobacillus* strain is used, thereby allowing the production of lactic acid and the lowering of pH in the feed. The resulting fermented feed contains a high abundance of the *Lactobacillus* strain that was used as inoculum, which is considered to elicit probiotic effects. This fermented feed is typically used as creep feed or as weaner feed and it would be interesting to study the effects of fermented feed on the composition of the GI tract microbiota and on development of the host immune system. Although the use of microbial feed interventions comes with challenges, such as added cost, labour and logistical requirements, they can be considered to be one of the most direct methods to steer the development of the gut microbiota.

7.9 Conclusions

Given that the sow's microbiota dictates to a certain extent the microbiota development of her offspring, it should be possible to steer the microbiota development of the piglets through the sow. Additionally, many predisposing risk factors can be eliminated in the first days after birth by providing adequate housing, climate control and prevention of cross-contamination from infected pigs. Steering development of the microbiota can be done as early as in the first weeks of postnatal life through dietary interventions. As summarized in this chapter, many studies have investigated the effects of dietary interventions after weaning, with mixed outcomes on composition of the microbiome. However, relatively few studies were conducted to look at dietary interventions during the suckling period. Although less practical to implement in production environments, the administration of dietary components during the suckling period might be more promising in terms of early-life stimulation of mucosal and systemic immune development, steering microbial succession along the GI tract and subsequent disease prevention.

To this end, both non-microbial and microbial feed interventions can steer the GI tract microbiota composition into the 'right' direction. This generally means a reduction in the relative abundance of Gram negative bacteria, such as proteobacteria, enterobacteria, *Salmonella*, coliforms, *E. coli*, and more specifically pathogens, such as ETEC and *S. suis*, although a decrease in *E. coli* diversity was also seen (Šmajš *et al.*, 2012). During the suckling period, an increase in the relative abundances of milk-degraders such as

lactobacilli and bifidobacteria is generally seen as a positive effect, while after weaning an increase in fibre-degraders such as *Prevotella* and clostridia is considered a positive sign of gut maturation. In several studies using a non-microbial feed intervention resulted in increased relative abundances of lactobacilli, bifidobacteria or strict anaerobes, and more specifically, some studies showed an increase in relative abundances of known probiotics (e.g. *Faecalibacterium prausnitzii*) after weaning. These are all considered as positive treatment effects. However, in a recent study it was found that when weaned piglets have a relatively high abundance of microbial members associated with suckling (*Lactobacillus* and *Oscillospira*), they are predisposed for infection by *Salmonella* (Argüello *et al.*, 2019).

Although some dietary fibres clearly confer beneficial health effects, recommendations for optimal inclusion rates are challenging (Pieper *et al.*, 2015). Similarly, adequate administration of probiotics or fermented products might be even more challenging, as living microbes must be stored and administered in such a way that they at least partly survive until they reach the piglet's GI tract. In this respect, the use of postbiotic, i.e. microbial fermentation components, might be a viable option, especially since they were shown to elicit responses similar to those of probiotics (Tsilingiri and Rescigno, 2012; Wegh *et al.*, 2019). Despite these challenges, pre- and probiotics are expected to play increasingly important roles in pig husbandry because of their potential to improve gut health and their potential to replace AGPs and other antimicrobial feed ingredients such as ZnO. The full potential of probiotics in pig husbandry has not yet been explored, as numerous porcine commensal bacteria remain uncultured and relatively few bacterial candidates have been tested for their positive effects on gut health as well as their safety as live microbial feed ingredients. One approach to finding promising candidates as probiotics in feed for piglets is by correlating growth/health traits with the abundance of particular microbial taxa. In one study, taxa such as *Christensenellaceae*, *Oscilibacter* and *Cellulosilyticum* were associated with a healthier host with increased feed efficiency (McCormack *et al.*, 2017). The potential of discovering new candidates for probiotic, or prebiotic use and for alternative compounds, such as essential oils and fungal supplements, is high because they have not all yet been tested in controlled *in vivo* trials. With regard to microbial and non-microbial feed interventions that have been tested to date, findings from many published experiments are highly promising. As a result, implementation of these feed interventions in pig husbandry is likely to increase in the future.

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