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## Review Article

## Non-antibiotic feed additives in diets for pigs: A review

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## ABSTRACT

A number of feed additives are marketed to assist in boosting the pigs' immune system, regulate gut microbiota, and reduce negative impacts of weaning and other environmental challenges. The most commonly used feed additives include acidifiers, zinc and copper, prebiotics, direct-fed microbials, yeast products, nucleotides, and plant extracts. Inclusion of pharmacological levels of zinc and copper, certain acidifiers, and several plant extracts have been reported to result in improved pig performance or improved immune function of pigs. It is also possible that use of prebiotics, direct-fed microbials, yeast, and nucleotides may have positive impacts on pig performance, but results have been less consistent and there is a need for more research in this area.

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## 1. Introduction

The increased awareness of potential negative effects of including antibiotic growth promoters in diets fed to pigs has resulted in an increased interest in producing pigs without using antibiotic growth promoters. However, by eliminating antibiotic growth promoters from diets fed to newly weaned pigs, disease problems may be increased and growth performance may be reduced. In contrast, removal of antibiotic growth promoters from diets fed to growing-finishing pigs does not always result in increased disease problems (Wierup, 2001). Because growth performance is increased to a greater extent in weanling pigs than in

growing – finishing pigs if antibiotic growth promoters are used, the negative effects on growth performance of removing antibiotics from diets fed to finishing pigs is less than for weanling pigs (Cromwell, 2013). Nevertheless, to avoid the negative effects of removing antibiotic growth promoters from diets for pigs, changes in management and nutritional strategies may be required (Pettigrew, 2006; Stein and Kil, 2006; Kil and Stein, 2010). The aim of these changes is to improve the pigs' ability to prevent pathogenic bacteria from colonizing the intestinal system, which often is accomplished via an improved immunological response to pathogens. This also can be accomplished via mechanisms that prevent the pathogens from adhering to intestinal mucosa or releasing toxins, and thus, reduce the damaging effects of the pathogens on the host. In the present contribution, some of the additives that may be used to ameliorate the negative impacts of removing antibiotic growth promoters from the diets will be discussed. The objective of the review is to provide an overview over the additives that are available for inclusion in diets for pigs and also to include the believed mechanisms for each additive as well as the most recent results from growth performance and digestibility experiments that are available. However, it is not the objective to provide an exhaustive review on each additive.

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## 2. Acidifiers

Acidifiers are often used as alternatives to antibiotic growth promoters because of their ability to create a favorable intestinal environment for beneficial microbes which may result in increased nutrient digestibility, increased growth performance, and reduced diarrhea. Dietary acidifiers may be organic or inorganic acids or salts of acids (Partanen and Mroz, 1999; Papatsiros and Billinis, 2012). Blends of acids including mixtures of various organic acids, or mixtures of organic and inorganic acids may also be used to maximize acidification effects in diets for pigs (Zentek et al., 2013; Ahmed et al., 2014; Kuang et al., 2015). However, despite many years of research, the exact mode of action of dietary acidifiers has not been fully elucidated, but the following mechanisms have been proposed: 1) a decreased or stabilized gastric pH may lead to increased pepsin activity; 2) a modulation and alteration of the gut microbiota may inhibit pathogenic bacterial activity; and 3) acidifiers may improve nutrient digestibility in the small and large intestines resulting in increased nutrient retention (Kil et al., 2011; Papatsiros and Billinis, 2012).

### 2.1. Organic acids

The most commonly used organic acids include formic acid, fumaric acid, lactic acid, and citric acids and a comprehensive review of the effects of these acids is available (Suiryarayana and Ramana, 2015). Supplementation of diets with organic acids usually results in a reduction in stomach pH (Eidelsburger et al., 1992b; Roth et al., 1992a; Radcliffe et al., 1998). Formic acid, citric acid, and benzoic acid improved growth rate and feed conversion ratio when included in diets fed weanling pigs (Guggenbuhl et al., 2007; Halas et al., 2010; Papatsiros et al., 2011; Diao et al., 2016; Luise et al., 2017) and growing pigs (Giesting and Easter, 1985; Suryanarayana et al., 2012). Formic acid and its salts had a positive effect on the apparent total tract digestibility (ATTD) of protein (Eckel et al., 1992), but not on ileal amino acid digestibility (Gabert et al., 1995) and in fact a reduction in ileal amino acid digestibility has been reported as a result of diet acidification (Gabert and Sauer, 1995). However, supplementation of diets with citric acid had a positive effect on the digestibility of protein, calcium and phosphorus in sows (Liu et al., 2014a, b). Dietary benzoic acid has been shown to improve the apparent digestibility of Ca and P in growing pigs (Sauer et al., 2009; Bühler et al., 2010; Xu et al., 2018), crude protein in weanling pigs (Guggenbuhl et al., 2007; Halas et al., 2010; Xu et al., 2018), and organic matter, crude protein, ether extract and crude fiber in sows (Kluge et al., 2010). Formic acid added to diets for weanling pigs may also increase intestinal microbial diversity and change concentrations of certain intestinal microbes (Luise et al., 2017). It is possible that sources of protein and inclusion levels of acids in the diets are among the reasons for the inconsistent results that have been reported (Blank et al., 1999; Kil et al., 2011). As an example, diets containing oilseed meals as the primary protein source usually show a greater response to acidifiers compared with diets containing milk protein and responses to acidifiers are usually dose dependent (Ravindran and Kornegay, 1993).

Recently, combinations of organic acids and medium-chain fatty acids have been demonstrated to reduce pathogenic activity than if acids or fatty acids are fed individually (Zentek et al., 2013) and the combination of organic acids and medium-chain fatty acids had positive effects on the digestibility of nutrients as well as on growth performance (Upadhaya et al., 2014; Kuang et al., 2015; Long et al., 2018). It was demonstrated that the reason for this observation is that expression of proinflammatory cytokines was downregulated and proliferation of lactobacillus was increased (Kuang et al., 2015). The pH in the stomach as well as the concentration of pathogenic

bacteria in the intestinal tract were also reduced in pigs fed diets containing a combination of organic acids and medium-chain fatty acids (Zentek et al., 2013). Likewise, the concentration of hydroxyl radicals in serum was reduced if pigs were fed a combination of organic acids and medium-chain fatty acids (Long et al., 2018).

### 2.2. Inorganic acids

Inorganic acids commonly used in diets include hydrochloric acid, sulfuric acid, and phosphoric acid. Positive responses to supplementations of diets with phosphoric acid and hydrochloric acid have been reported (Mahan et al., 1996), although a lack of a positive response to hydrochloric acid has also been observed (Gedek et al., 1992; Eidelsburger et al., 1992a; Roth et al., 1992b; Kil et al., 2006, 2011). Supplementation of diets with sulfuric acid has been reported to result in a negative effect on growth performance (Eidelsburger et al., 1992b; Mahan et al., 1996).

### 2.3. Salts of acids

Salts of acids also have been used as acidifiers for pigs and these salts include calcium-formate, potassium-diformate, sodium-diformate, and sodium-fumarate. Positive effects of calcium-formate on growth performance and diarrhea scores were reported (Bosi et al., 2007), but in general, calcium-formate is not as efficient as potassium-diformate (Li et al., 2008a). Potassium-diformate may result in greater gastric hydrochloric acid and lactic acid concentrations because of increased mRNA expression of H<sup>+</sup>-K<sup>+</sup>-ATPase and gastrin receptors in the gastric oxyntic mucosa (Xia et al., 2016). Positive effects on dry matter and protein digestibility of supplementing diets with sodium-diformate and sodium-fumarate have also been reported (Eidelsburger et al., 1992b).

Butyric acid, also known as butanoic acid, is one of the short-chain fatty acids (SCFA) that are produced by microbial fermentation in the gastrointestinal (GI) tract of pigs (Mallo et al., 2012). Especially, the propionic and butyric acids produced in the GI tract are considered important metabolites that have antibacterial effects on pathogenic bacteria (Stecher and Hardt, 2011). Addition of butyric acid directly to a swine diet may be limited because of its highly volatile and corrosive characteristics (Piva et al., 2002). Therefore, some products of butyric acid have been used in combined forms with Ca and Na. Machinsky et al. (2015) observed a positive effect of Na-butyrate on the protein digestibility of pigs. Recently, dietary Na-butyrate decreased diarrhea scores of weanling pigs (Fang et al., 2014) and improved growth performance (Lu et al., 2008; Hanczakowska et al., 2014; Huang et al., 2015). In addition, Na-butyrate supplementation to gestating sow diets and pre-weanling pig diets (Le Gall et al., 2009; Lu et al., 2012) was reported to have a positive effect on muscle and adipose tissue oxidative genes and growth performance.

### 2.4. Blends of acids

A mixture of citric acids and sorbic acids resulted in improved growth performance of pigs (Grilli et al., 2010), and a blend of organic and inorganic acids increased fecal *Lactobacillus* spp. and decreased *Escherichia coli* counts, but did not improve growth performance of pigs (Ahmed et al., 2014). Likewise, addition of a blend of organic acids (fumaric, lactate, citric, propionic, and benzoic acids) followed by a blend of phosphoric, fumaric, lactic, and citric acid improved growth performance of newly weaned pigs (Walsh et al., 2007). Upadhaya et al. (2014) also reported increased *Lactobacillus* spp. count in feces from pigs fed a blend of acidifiers and a blend of acidifiers was effective in reducing diarrhea in pigs that were stressed by changing temperatures (Wang et al., 2016a, b).

In conclusion, a number of acidifiers are used in diets fed to pigs, but results reported in the literature have not been consistently positive. Further research is, therefore, needed to clarify the modes of action of acidifiers and establish under which conditions a positive response can be expected.

### 3. Minerals

Minerals are inorganic elements needed by pigs for maintenance, growth, and reproduction. Minerals needed in quantities greater than 100 mg/kg of feed are called macro minerals whereas minerals required in smaller quantities are called micro minerals or trace minerals. Examples of micro minerals are Cu and Zn, which are needed for normal bodily functions of pigs. However, unlike most other minerals, Cu and Zn have antimicrobial properties and they are therefore often added to diets in quantities greater than what is needed to fulfill the nutritional requirements.

#### 3.1. Zinc

Zinc is a component and activator of several metalloenzymes, and has a major function in production and secretion of hormones. It also plays a role in skin and wound healing and in the integrity of the immune system (McDowell, 1992). Nursery pigs usually require 80 to 100 mg/kg of Zn (van Heugten et al., 2003; NRC, 2012) and deficiency of Zn in weanling pig diets leads to growth retardation, loss of appetite, skeletal abnormalities, and hyperkeratinization of the skin called parakeratosis (Ku et al., 1970; Prasad et al., 1971). However, use of pharmacological levels (2,000 to 4,000 mg/kg) of inorganic Zn in the form of ZnO is a common recommendation to reduce post-weaning diarrhea and improve growth performance (Poulsen, 1998; Smith et al., 1997; Hill et al., 2000; Hu et al., 2012). It has also been reported that high levels of Zn stimulate and can improve feed intake by 14% to 17% (Hahn and Baker, 1993; Case and Carlson, 2002). Aside from ZnO, there are also other forms of Zn, which can be included in diets at lower concentrations. These forms include the chelated sources of Zn such as Zn-methionine, which has greater bioavailability of Zn than ZnO (Ward et al., 1996) and addition of 250 mg/kg of Zn-methionine to nursery diets has beneficial effects that are equivalent to addition of 2,000 mg/kg of Zn from ZnO (Mavromichalis et al., 2001). The biological mechanism of Zn in enhancing growth performance points may be related to its function in the intestinal integrity and morphology in weanling pigs (Pearce et al., 2015). High Zn intake improves the intestinal morphology of weaning pigs, increasing the villous height and the villous height to crypt depth ratio (Carlson et al., 1998; Li et al., 2001, 2006; Hu et al., 2013a; Xia et al., 2017; Zhu et al., 2017) and decreases crypt depth in the small intestine of weaned pigs (Li et al., 2001; Zhu et al., 2017). Dietary Zn also assists in regeneration of injured intestinal epithelial tissue (Alam et al., 1994), stability of the microflora and diversity of the coliform microbes (Katouli et al., 1999), reduction of intestinal permeability of weanling pigs (Zhang and Guo, 2009), and lymphocyte proliferation (van Heugten et al., 2003).

The intestinal barrier is mainly formed by a layer of epithelial cells joined by tight junction proteins, which consist mainly of the transmembrane protein complexes (e.g., claudins and occludins) and the cytosolic proteins ZO (e.g., junctional adhesion molecule, ZO-1, ZO-2 and ZO-3; Al-Sadi et al., 2009). The ZO-1 and occludin, therefore, are key proteins of tight junction, and the levels of these proteins are consistently associated with the gut barrier function (Song et al., 2015). High levels of Zn intake increase the ileum mucosa (Zhang and Guo, 2009; Zhu et al., 2017) and jejunum mucosa (Hu et al., 2013a; Xia et al., 2017) of occludin and ZO-1 and high dietary Zn reduces the paracellular flux of FD4 across the

epithelium (Hu et al., 2013a; Song et al., 2015), which is an indication of improved intestinal barrier function.

Zinc is also a molecular signal for immune cells, and it is required for differentiation and generation of T helper cells (Prasad, 2014). Deficiency of Zn induces thymic atrophy, lymphopenia, and compromised cell- and antibody-mediated responses, resulting in increased rates and longer duration of infection (Hirano et al., 2008). In contrast, high levels of Zn downregulate the mRNA expression of pro-inflammatory cytokines: *TNF- $\alpha$* , *IFN- $\gamma$* , *IL-6*, *IL-8*, *IL-1 $\beta$*  and nuclear factor kappa beta (Hu et al., 2013a; Zhu et al., 2017), and upregulate the anti-inflammatory cytokine *TFG- $\beta$*  (Zhu et al., 2017). Pro-inflammatory cytokines (e.g., *TNF- $\alpha$* , *IFN- $\gamma$* , *IL-1 $\beta$*  and *IL-6*) may induce pathological openings of the intestinal tight junction barrier and increase intestinal epithelial permeability (Al-Sadi et al., 2009), which will disrupt intestinal barrier function (Hu et al., 2013b). High doses of Zn doses may also increase mRNA expression of *IGF-1* and *CDK4* (Xia et al., 2017). The IGF-1 can be locally synthesized in the gastrointestinal tract, is an important mediator of the proliferation and differentiation of enterocytes (Prasad, 2014), whereas CDK4 is used as a marker to monitor proliferation and apoptosis due to its functions in G1 phase of cell cycle (Hu et al., 2013b). Also, high Zn levels in the diet improved the small intestinal redox state in weaned piglets by increasing the ratio of reduced glutathione to oxidized glutathione and prevented apoptosis in the small intestine and increased the number of goblet cells in the intestinal tract (Slade et al., 2011).

However, addition of pharmacological levels of ZnO in diets for weanling pigs will reduce the digestibility of Ca and P and reduce the effectiveness of microbial phytase in the diets (Walk et al., 2013, 2015; Blavi et al., 2017). The reason for the reduced digestibility of P is most likely that an antagonistic relationship between Zn and P exists, whereas reduced digestibility of Ca is a result of competition for the absorptive transporters in the brush border (Bertolo et al., 2001; Walk et al., 2015).

Inclusion of pharmacological levels of ZnO will be discontinued in the European Union from 2022 due to concerns about buildup of Zn in soil fertilized with pig manure. However, in the rest of the world, ZnO may still be used.

#### 3.2. Copper

Copper is an essential component of several metalloenzymes including cytochrome oxidase and lysyl oxidase, and is involved in oxidation–reduction reactions, transport of oxygen and electrons, and protection against oxidative stress (Hill, 2013). Copper is also involved in metabolic reactions, including cellular respiration, tissue pigmentation, hemoglobin formation, and connective tissue development (McDowell, 1992). Neonatal pigs usually require 5 to 6 mg/kg of Cu for normal metabolism (Underwood, 1977; NRC, 2012), but as pigs get older, the requirement for Cu decreases. Pigs deprived of copper develop critical dysfunctions and hypocuprosis (Suttle, 2010). Microcytic anemia is a sign of copper deficiency due to its role in hemoglobin formation and development (Hart et al., 2002; Suttle and Angus, 1978). Pigs may also suffer from bone abnormalities and unusual leg condition with various degrees of crookedness due to a lack of copper in the diet (Baxter et al., 1953). Addition of pharmacological levels of Cu in pig diets has been a common practice to improve growth performance (Ma et al., 2015), and supplementing Cu to diets fed to weanling pigs at 100 to 250 mg/kg may reduce post-weaning scouring and improve average daily gain (ADG) and average daily feed intake (ADFI) (Poulsen, 1995; Rutkowska-Pejsak et al., 1998; Perez et al., 2011). In most cases, additional Cu is included in the sulfate form, however, other chemical forms of Cu may be used. It has been reported that tribasic copper chloride (TBCC) is as effective as  $\text{CuSO}_4$  in enhancing

growth rate and feed efficiency in pigs (Cromwell et al., 1998). Use of TBCC instead of CuSO<sub>4</sub> also results in improved utilization of P in broilers (Banks et al., 2004), which may be because TBCC results in less inhibition of microbial phytase (Pang and Applegate, 2006). It also appears that TBCC is less aggressive in premixes, and therefore reduces the destruction of vitamins, phytase, and probiotics in vitamin mineral premixes during storage. Chelated Cu, such as Cu citrate, may also be used in diets for pigs, and because the availability of Cu in Cu citrate is greater than in CuSO<sub>4</sub>, lower concentrations are needed in the diets which results in reduced excretion of Cu (Armstrong et al., 2004).

The growth promoting effects of dietary Cu have been attributed to its bacteriostatic and bactericidal properties (Stahly et al., 1980) because Cu may reduce bacterial populations in the intestine, which may affect the growth and community structure of microorganisms in the cecum and colon (Hojberg et al., 2005). It has also been speculated that improvement in ADFI as a result of Cu supplementation is due to the role of Cu in upregulating the mRNA expression of neuropeptide Y (Li et al., 2008b), which is considered a feed intake inducer (Gehlert, 1999). Copper may also disrupt enzyme structure and functions of bacteria by binding to S or carboxylate-containing groups and amino groups of proteins (Sterritt and Lester, 1980). Inclusion of Cu at pharmacological levels in diets fed to weanling pigs also increases villus height and reduces crypt depth, thus improving intestinal health (Zhao et al., 2007).

### 3.3. Additive effects of Cu and Zn

Results of several experiments have indicated beneficial effects of supplementing pharmacological levels of Cu and Zn individually, but in some experiments, effects of adding pharmacological levels of both Cu and Zn were investigated. However, results of some experiments indicated that effects of the two minerals are not additive (Hill et al., 2000), but other results have indicated some additive effects (Perez et al., 2011). It was reported that ZnO may modify the colonic microbial profile, whereas Cu in addition to altering the microbiota profile also reduces microbial diversity in the ileum and colon, which may be the reason for additive effects of the two minerals (Namkung et al., 2006).

## 4. Probiotics

The composition of the gut microbiota plays an important role in the health of pigs and increasing the population of bacteria of genus Bifidobacterium, lactobacilli, and eubacteria in the gut may improve the health of the animals, and decrease the risk of diseases (Roberfroid et al., 2010; van der Aar et al., 2017). Growth of these bacteria increases the concentration of lactic and acetic acid, which will reduce the pH in the intestine and increase fermentation with a subsequent increase in the concentration of SCFA and a reduced concentration of pathogenic bacteria (Smiricky-Tjardes et al., 2003).

Probiotics are mainly non digestible oligosaccharides and have been defined as “non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” (Gibson and Roberfroid, 1995). Non-digestible carbohydrates are the main substrate for gut microbiota, however, only a fraction of non-digestible carbohydrates can be considered as prebiotic because not all indigestible carbohydrates impacts the microbial population.

Inulin, fructo-oligosaccharides, transgalacto-oligosaccharides, and lactulose are the most common carbohydrates that have been recognized as prebiotics because these carbohydrates are easily fermentable and therefore will result in reduced luminal pH (de Lange et al., 2010; Bach Knudsen et al., 2012). However, other

dietary carbohydrates such as arabino-xylans, xyloglucans, and resistant starch also may have prebiotic effects (Bach Knudsen et al., 2012). Prebiotics also may be obtained by chemical processing that hydrolyze polysaccharides (i.e., isomalto-oligosaccharides from starch) or by enzymatic or chemical synthesis from disaccharides (Broek et al., 2008). Most prebiotics are synthesized or isolated from plant and algae polysaccharides (Smiricky-Tjardes et al., 2003; Saad et al., 2013; Wu et al., 2017).

Beneficial effects of prebiotics in diets fed to pigs have been related to increased fermentability, because the subsequent synthesis of SCFA results in reduced intestinal pH. Increased concentrations of SCFA also reduce protein fermentation in the intestinal tract (Awati et al., 2006; Roberfroid et al., 2010; Lindberg, 2014). Butyrate regulates epithelial cell growth and induces differentiation and apoptosis in the small intestine, which is believed to result in increased intestinal cell proliferation and improved digestive and absorptive capacities of the small intestine (Lindberg, 2014; van der Aar et al., 2017). Addition of 100 or 200 mg/kg of chito-oligosaccharide (a derived of the of chitosan) to diets for weaning pigs, improved growth performance and increased digestibility of dietary nutrients, decreased the incidence of diarrhea, and improved small intestine morphology (Liu et al., 2008). Isomalto-oligosaccharides, added at 6.0 g/kg to diets for weanling pigs improved growth performance, decreased fecal scores and increased ATTD of dry matter, organic matter, and gross energy in diets fed to weanling pigs. Also, greater villus height in the ileum and increased concentrations of volatile fatty acids in cecum and colon contents were observed by adding isomalto-oligosaccharides to diets (Wang et al., 2016a, b; Wu et al., 2017). However, supplementation of 0.1% of chicory, mannan oligosaccharides, or 0.02% of chitosan to diets for weaning pigs had no effect on growth performance or serum concentration of IgA (Li et al., 2016) and addition of inulin to diets for weanling pigs had not effect on ATTD of gross energy or dry matter or on nitrogen retention (Acosta et al., 2017).

Results of several studies have also demonstrated that the prebiotic lactulose may result in greater concentrations of serum IgM and IgA and improved immunity against *Salmonella typhimurium* (Naqid et al., 2015). Positive effects of lactulose on growth performance, microbiota composition, and immune response of weaning pig challenged with enterotoxigenic *E. coli* K88 or *S. typhimurium* have also been reported, but those effects have not been observed in growing pigs (Guerra-Ordaz et al., 2014; Naqid et al., 2015; Tran et al., 2016). Likewise, increased cell-mediated immune response, *IL-1β* gene expression, and serum levels of IL-1β, IL-2, and IL-6, were observed when diets for weaned pigs were supplemented with chitosan [6-sugar unit of N acetyl glucosamine with β-(1 to 4) linkages] and galacto-mannan oligosaccharides (Yin et al., 2008).

In conclusion, although several mechanisms have been suggested for the action of prebiotics, it appears that modified intestinal microbiota and increased concentrations of volatile fatty acids in the intestinal tract are the best documented and accepted effects of prebiotics. However, it is possible that prebiotics also impact the immune system, but additional research is needed to document these effects. Among the compounds with prebiotic effects, inulin and fructo oligosaccharides are the most studied. The inconsistent results that have been obtained with these compounds may be due to differences in age of pigs, health status, or environmental conditions.

## 5. Direct-fed microbials (DFM) and yeast

Direct-fed microbials, which may be more commonly known as probiotics, are defined as, “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001).” Since 1989, the Food and Drug



Administration has required that the term probiotic only be used when referring to human microbial products; therefore, the term “DFM” is used in the U.S. feed industry, whereas “probiotic” is used interchangeably with human and animal feed worldwide. Direct-fed microbials are categorized into 3 main groups: *Bacillus*, lactic acid-producing bacteria, and yeast (Stein and Kil, 2006; NRC, 2012). Yeast include a broad range of products that may be available in pig feed, therefore, a brief overview of yeast will be separately discussed in the current review. *Bacillus*-based DFM are spore-forming, which makes them thermostable and able to survive at low pH. *Bacillus*-based DFM have been identified as potent producers of extracellular fiber-degrading enzymes, which may aid nutrient digestion and utilization (Ferrari et al., 1993; Schreier, 1993). Lactic-acid producing bacteria are not spore-forming and survival during feed processing is of concern (de Lange et al., 2010). Lactic-acid producing bacteria dominate the gastrointestinal tract of the nursing pig (Li et al., 2003; Richards et al., 2005), which helps reduce the pH in the gut by producing lactic acid through fermentation, inhibiting enteric pathogens (Vandenbergh, 1993), and improving host immunity (Niers et al., 2005; de Lange et al., 2010). However, after weaning of pigs, the concentration of lactic-acid producing bacteria diminishes; therefore, supplementation of weaned pig diets with lactic-acid producing DFM may be beneficial (Stein and Kil, 2006).

Addition of DFM to swine diets may improve gut health by modifying the microflora, which may help control pathogens (Prescott et al., 2005), enhance immune regulation and response (Galdeano and Perdigon, 2006), increase nutrient digestibility (Giang et al., 2011), improve health status, and improve pig growth performance (Kenny et al., 2011; Cromwell, 2013). Addition of DFM to diets for pigs may also reduce the immune stimulation, indicated by a reduction in pro-inflammatory cytokines in enterocytes, which may shift energy normally used for excessive immune stimulation to growth, thereby improving feed efficiency (Cho et al., 2011).

### 5.1. Mode of action

As the name suggests, DFM are added to the diet where they must survive processing technologies such as extrusion and pelleting. Once consumed by the pig, DFM enter the stomach where they are subjected to a low pH and pepsin. *Bacillus* DFM are metabolically inactive spores that are thermostable and survive at a low pH and, therefore, are thought to survive feed processing and digestion in the stomach. The pH in the small intestine is 6 to 7, which is optimal for the spores to germinate, grow, and produce enzymes (Merchant et al., 2011). The DFM continue to survive due to their ability to produce enzymes that degrade the feed and produce SCFA as a by-product of fermentation. The SCFA produced are utilized by the pig as an energy source, and the increased SCFA concentration reduces the pH in the gastrointestinal tract, which may inhibit growth of pathogenic bacteria. The DFM also may degrade non-starch polysaccharides to reducing sugars that may serve as an energy source for the pig (Jaworski et al., 2017). Direct-fed microbials may also improve gastrointestinal health by promoting the growth of beneficial bacteria such as lactobacilli and bifidobacteria, thereby decreasing the growth of deleterious bacteria from the large family of Gram-negative Enterobacteriaceae. The decrease in pathogenic bacteria and increase in gastrointestinal health may correspond to an increase in the ability of the pig to digest and ferment nutrients, enhance their utilization of feed and energy, decrease the maintenance energy requirement associated with immune system stimulation, and thereby increase growth performance (Kenny et al., 2011).

### 5.2. Efficacy of direct-fed microbials

Published reviews have concluded that the efficacy of DFM added to swine diets is inconsistent (Pollmann, 1986, 1992; Nousiainen and Setälä, 1993; Stavric and Kornegay, 1995). However, due to improvements in the strains used in commercial DFM, older reports may not always be appropriate to use to assess the efficacy of new DFM because the development of DFM and the technology associated with production of DFM has improved, which may lead to increased efficiency.

Addition of 0,  $5.0 \times 10^4$ ,  $6.7 \times 10^6$ , or  $7.5 \times 10^8$  cfu/d of *Bifidobacterium globosum* A (lactic-acid producing DFM) to weanling pig corn-soybean meal-based diets quadratically improved ADG and average daily feed intake, but did not affect G:F, immune response, or pH of intestinal contents (Apgar et al., 1993). This same feeding regimen was maintained through the growing-finishing phase and pig performance and carcass characteristics were not affected by DFM addition (Apgar et al., 1993). More recent studies utilizing lactic-acid producing DFM (*Bifidobacterium lactis* NCC2818) supplemented to a weanling pig diet produced an up-regulation of proteins associated with epithelial tight cell junctions and reduced IgA in intestinal mucosal tissues, indicating increased gut barrier function (Lewis et al., 2013). It has also been demonstrated that effects of DFM are most likely diet dependent (Merrifield et al., 2013; Bailey, 2016).

Feeding DFM has been suggested to increase cellulolytic bacteria in the rumen of cows (Dawson et al., 1990) and to enhance dietary fiber fermentation in the horse (Godbee, 1983). However, addition of 0, 8%, or 16% peanut hulls, added at the expense of corn, to diets fed to pigs linearly reduced the ATTD of dry matter, acid detergent fiber, and neutral detergent fiber, and DFM addition did not ameliorate the reduced digestibility (Kornegay et al., 1995). Kornegay and Risley (1996) observed no difference in the ATTD of dry matter, acid detergent fiber, and neutral detergent fiber by 60 kg pigs fed either a corn-soybean meal diet without or with a DFM containing *Bacillus subtilis* and *Bacillus licheniformis*, or with a DFM containing *B. subtilis*, *B. licheniformis*, and *Bacillus pumilus*.

In a study utilizing 270 wean-to-finish pigs, the dose of DFM (0,  $0.64 \times 10^6$ ,  $1.28 \times 10^6$ ,  $1.92 \times 10^6$  viable spores of BioPlus 2B, which contained *B. licheniformis* and *B. subtilis* in a 1:1 ratio) and duration of DFM addition (weaning only or wean-to-finish) to pig diets was investigated. Results indicated that ADG, G:F, and carcass quality were improved with increased dose and duration of DFM addition (Alexopoulos et al., 2004). Lee et al. (2014) produced a *B. subtilis* DFM grown on citrus-juice waste and included this DFM at 0, 1.5, 3.0, or 4.5 g/kg in phase 1 and phase 2 corn-soybean meal based nursery pig diets. Linear improvements were observed in pig growth performance, ATTD of nutrients and energy, serum immunoglobulins, and small intestinal morphology (Lee et al., 2014).

Improved ADG and G:F and reduced time required to wash manure off of mats was observed by addition of 0.05% DFM comprised of two strains of *B. licheniformis* and one strain of *B. subtilis* (Davis et al., 2008). The authors hypothesized that performance and pen cleaning were improved with DFM addition because of increased dietary fiber degradation by enzymes secreted by the DFM. Therefore, further research is necessary to determine the effect of *Bacillus*-based DFM on dietary fiber fermentation. The ATTD of N and energy by pigs fed a corn-soybean meal-based diet were improved by addition of a DFM composed of *B. subtilis* and *Clostridium butyricum* and, subsequently, pig ADG and G:F were improved (Meng et al., 2010). Pigs challenged with *Salmonella enterica* had reduced ADG and G:F and increased bacterial shedding scores compared with non-challenged pigs, but addition of a *Lactobacillus plantarum* DFM did not influence recovery from the challenge (Geburu et al., 2010). Weanling pigs fed a *Lactobacillus*

*reuteri* and *L. plantarum* DFM for 28 d had improved overall ADG and ATTD of protein and gross energy compared with pigs fed no DFM. Results for pigs fed the DFM were similar to results for pigs fed a diet containing 0.01% apramycin, indicating that the *Lactobacillus reuteri* and *L. plantarum* DFM may minimize antibiotic use in weanling pig diets (Zhao and Kim, 2015). However, DFM cannot replace antibiotics in terms of preventing or treating of sickness or disease, but seem to be a viable alternative to antibiotics used as growth promoters.

A diet containing corn, soybean meal, and distillers dried grains with solubles (DDGS) supplemented with 500 g/metric ton *Bacillus* spp. DFM and fed to nursery pigs had a 100 kcal/kg increase in digestible energy (DE) due to a 9.2% increase in the ATTD of neutral detergent fiber compared with the control diet with no DFM (Owusu-Asiedu et al., 2014). Growing-finishing pigs fed high-fiber diets based on corn, soybean meal, DDGS, wheat middlings, corn germ, and soybean hulls supplemented with a *Bacillus* spp. DFM had increased fecal SCFA concentrations and, subsequently, greater available dietary energy, which corresponded with improved ADG and G:F, and a greater loin eye area and fat-free lean percentage compared with pigs fed no DFM (Jaworski et al., 2014). Likewise, improved G:F of weanling pigs has also been reported as a result of inclusion of DFM in the diets (Jaworski et al., 2017). These results are supportive of the hypothesis that *Bacillus* DFM may result in increased fiber degradation in the intestinal tract of pigs. A consequence of this is that the response to DFM most likely is diet dependent because not all diets contain the same amount and types of fibers. In addition, addition of a DFM to diets for pigs may result in increased concentrations of intestinal microbes that express enzymes that aid in fiber fermentation.

### 5.3. Yeast

Yeast may be supplemented in diets fed to pigs in several forms: whole live yeast cells, heat-treated yeast cells, ground yeast cells, purified yeast cell cultures, and yeast extracts. Effects of the supplements vary based on the form of supplementation, and therefore, the intended uses of each type of supplementation also vary. As such, care must be taken in the interpretation of results of various yeast supplements. However, there are some generally proposed uses for yeast supplements. Yeast or yeast-based product supplementation may boost ADFI and pig growth performance, augment mucosal immunity, promote intestinal development, adsorb mycotoxins, reduce post-weaning diarrhea, and modulate gut microbiota (Kogan and Kocher, 2007; Shen et al., 2009; Sauer et al., 2011; Jiang et al., 2015). Substantial evidence, however, for many of these claims has yet to be reported.

Yeast is resilient to deactivation in the gut and may be effective in modulating colonization of the host's gut (Bontempo et al., 2006). The mechanism by which yeast cells can provide these benefits is the result of the composition of the yeast cells. The specific sugar types that form large parts of the cell walls of yeast, notably  $\beta$ -D-glucans and  $\alpha$ -D-mannans, are believed to be responsible for the effects of yeasts cells (Kogan and Kocher, 2007; Shen et al., 2009).

Additionally, yeast extracts contains nucleotides, and although nucleotides are present in nearly all feed ingredients and the requirement for nucleotides is typically met via endogenous synthesis (Sauer et al., 2011), there is some evidence that the requirement for nucleotides may be increased under certain conditions, such as during disease, high stress, or rapid growth (Carver and Walker, 1995; Waititu et al., 2016). If there was a period of a pig's life that matched these conditions, and therefore induced a potential nucleotide deficiency, it would be during the time right after weaning, and in that case, yeast cells may be potentially beneficial.

Part of easing the transition around weaning of pigs involves preparing the gut for the change in diet form. Weaning itself causes villus atrophy and decreases replacement of enterocytes both of which inhibit nutrient absorption (Jiang et al., 2015). However, supplementation of live yeast and finely ground live yeast may result in increased villus heights and villus-to-crypt ratios (Bontempo et al., 2006; Jiang et al., 2015). Similar results were reported for yeast fermentation products (Shen et al., 2009). Additionally, there has been evidence that live yeast supplementation may increase gut cell proliferation and increase the concentration of glycoconjugates in the mucin of supplemented pigs, thereby increasing their resistance to pathogenic invasion (Bontempo et al., 2006). However, supplementation with yeast cultures or yeast culture products supplemented with mannan-oligosaccharides has also been shown to have no benefits on gut morphology (van der Peet-Schwering et al., 2007).

Many of the beneficial effects of yeast products are claimed to be the result of immune-modulation (Kogan and Kocher, 2007; Molist et al., 2014) and there is evidence that yeast and yeast products stimulate the immune system because the  $\beta$ -D-glucans in yeast cell walls enhance the function of macrophages and neutrophils by binding to their receptors causing cascades cytokines and increased antibody production (Kogan and Kocher, 2007; Kim et al., 2017). Feeding of live yeast and finely ground live yeast also increases serum concentrations of IgA, IL-2, and IL-6 (Jiang et al., 2015). The  $\alpha$ -D-mannans in yeast have possible antioxidant effects (Kogan and Kocher, 2007). In particular, the  $\alpha$ -D-mannans are believed to bind to mannose-specific receptors that are present on many bacteria such as *E. coli* and *Salmonella* spp., which prevents adhesion of these pathogens to the mannose rich glycoproteins lining the intestinal lumen (Kogan and Kocher, 2007). Indeed, it was reported that pigs that were fed diets supplemented with live yeast and exposed to enterotoxigenic *E. coli* had reduced disease-related stress, reduced diarrhea scores, reduced duration of diarrhea, and reduced shedding of *E. coli* (Trckova et al., 2014). Likewise, pigs that are challenged with *E. coli* K88 and fed diets containing a yeast fermentation product have greater concentrations of serum tumor necrosis factor-alpha, less diarrhea, greater appetite and reduced adherence of *E. coli* to the intestinal mucosa than pigs fed an un-supplemented control (Kiarie et al., 2011, 2012). However, results of other studies were less affirmative (Weedman et al., 2011).

Results from research investigating growth performance of pigs fed yeast or yeast-based products are mixed, but it has been reported that there were no difference between pigs fed antibiotic growth promoters and pigs fed diets supplemented with yeast on growth performance, nutrient digestibility, and intestinal morphology, indicating that yeast products may be an effective alternative to antibiotic growth promoters (Shen et al., 2009). It also was demonstrated that the immune-potentiating costs of yeast-based supplements had no negative effect on pig growth performance (Molist et al., 2014). However, it has also been reported that yeast cultures have no impact on pig growth performance or ATTD of dry matter, crude protein, acid detergent fiber, and neutral detergent fiber (Kornegay et al., 1995).

In conclusion, DFM supplementation to swine diets has produced more beneficial results in the past decade compared with earlier reports, indicating an improvement in the development and use of DFM. Lactic acid-producing bacteria appear to be more beneficial for weanling pigs to help stabilize the gastrointestinal tract after weaning, whereas *Bacillus*-based DFM may be more beneficial for growing-finishing pigs to increase the digestibility of energy and nutrients in less expensive high-fiber diets and, subsequently, increase performance and carcass characteristics. Effects of supplementing pig diets with yeast products is not fully understood, but it is believed that yeast products may have a number of

positive effects if added to diets for pigs and there is some evidence that yeast-based products may be used as partial or fully substitutes for antibiotic growth promoters. Intestinal health benefits and immunomodulatory effects of yeast-cells are significant and represent the most likely benefits of addition of yeast to diets for pigs.

## 6. Nucleotides

Nucleotides are molecules that contain a nitrogenous base, which is a purine or a pyrimidine, linked to a pentose sugar and at least one phosphate group. In ribonucleic acids (RNA), the pentose sugar is a ribose whereas the ribose in deoxyribonucleic acid (DNA) is a 2'-deoxyribose. Pyrimidines include uridine, cytosine, and thymine and they all have a six membered ring structure. Purines include adenine, guanine, and hypoxanthine and they contain a six membered ring structure that is attached to a five membered ring. The phosphate group may be in a mono, di, or tri phosphate form, and is commonly esterified to the C-5' hydroxyl group of the pentose sugar (Rudolph, 1994). A chain of nucleotides attached together via a phosphodiester linkage at the 3' and 5' positions of neighboring ribose units are called polynucleotides or nucleic acids. Nucleosides are similar in structure to nucleotides with the exception that the phosphate groups are absent.

Nucleotides are synthesized by the animals via the *de novo* Pathway or the Salvage Pathway, but *de novo* synthesis requires the amino acid glutamine and is considered a metabolically costly process as it requires energy in the form of adenosine tri phosphate. The salvage pathway synthesizes nucleotides by using products of nucleotide catabolism or from dietary sources. In addition to being building blocks for nucleic acids (DNA and RNA), nucleotides also have physiological roles in the body such as being a source of energy, cofactors in oxidation and reduction reactions, serve as physiological regulators, and carry activated intermediates (i. e., UDP-glucose, CMP-sialic acid, and CDP-choline) and acyl groups (i. e., CoA). In addition, nucleotides may influence the development of the immune system, the microbiota of the intestinal tract, and the integrity of the small intestine.

Dietary nucleotide supplementation has been associated with both humoral and cellular immunity, but the exact mechanism has not been elucidated. Dietary nucleotides contribute to the circulating pool of nucleosides available to stimulate leukocyte production (Kulkarni et al., 1994; Carver and Walker, 1995). Therefore, there is an elevated need for nucleotides during periods of immunological challenges. Supplementation of diets with nucleotides may increase the number of lymphocytes and macrophages of the intra-epithelium of the piglet ileum (Domeneghini et al., 2004; Šperanda et al., 2008), decrease damage of the blood lymphocyte DNA (Salobir et al., 2005), decrease the concentration of TNF- $\alpha$  and IL-6 in blood serum 2 and 4 h after an *E. coli* injection (Hung, 2015), and increase plasma and serum concentrations of IgA (Lee et al., 2007; Sauer et al., 2012a, b) in pigs.

Dietary nucleotides enhance intestinal absorption of iron, affect lipoprotein and long chain polyunsaturated fatty acid metabolism, have trophic effects on the intestinal mucosa and liver, and reduce the incidence of diarrhea (Cosgrove, 1998; Schlimme et al., 2000). The fecal microbiota of human infants fed a nucleotide-supplemented commercial milk formula had a predominance of bifidobacteria (Tanaka and Mutai, 1980), whereas enterobacteria dominated in the fecal microbiota of infants fed a commercial formula without nucleotide supplementation (Uauy, 1994). These data indicate that nucleotide supplementation may positively influence the microbiota in the gastrointestinal tract which leads to a lowering of gastric pH and hinders proliferation of pathogenic bacterial species as evidenced by a lower rate of diarrhea (Yu, 1998).

Newly weaned pigs fed a nucleotide deficient diet supplemented with nucleosides had elevated quantities of probiotic bacteria and reduced concentrations of *C. perfringens* compared with control pigs fed non-supplemented diets (Mateo et al., 2004). Pigs infected by *E. coli* that were fed diets supplemented with 4% yeast extract as a source of nucleotides had ADG and G:F, and reduced incidence of diarrhea (Maribo, 2003). However, it has also been reported that nucleotide supplementation has no impact on the composition of the microbiota in the small and large intestine of piglets (Sauer et al., 2011, 2012a, b).

Dietary nucleotides may enhance the growth and maturation of intestinal epithelial cells as evidenced by an increased formation of mucosal protein, DNA, taller villi in the small intestine, and increased maltase to lactase enzyme ratio (Uauy et al., 1990; Carver, 1994). Dietary nucleotides may also stimulate enterocyte differentiation (Sanderson and He, 1994). Parenteral supplementation of nucleic acids supports mucosal cell proliferation and function as demonstrated by increased mucosal wet weight, protein and DNA contents, villous height, and narrower tight junctions of the jejunal mucosa width (Kishibuchi et al., 1997; Tsujinaka et al., 1999).

Improvement in growth performance was observed when pure nucleotides were supplemented to diets fed to pigs (Zomborszky-Kovacs et al., 2000; Weaver and Kim, 2014), or if a nucleotide-rich yeast extract was used (Carlson et al., 2005; Waititu et al., 2016). However, results of several other studies indicated that supplementation of pig diets with nucleotides had no influence on growth performance (Di Giancamillo et al., 2003; Domeneghini et al., 2004; Lee et al., 2007; Martinez-Puig et al., 2007; Šperanda et al., 2008; Moore et al., 2011; Sauer et al., 2012a,b; Waititu, 2016). It is possible that these inconsistent responses to nucleotide supplementation are a result of differences in the amount and type of nucleotides provided and the existing environmental condition (Grimble and Westwood, 2001; Sauer et al., 2011; Hung, 2015; Waititu, 2016).

## 7. Plant extracts

Plant extracts are secondary plant metabolites, which are responsible for the odor and color of plants. Plant extracts are composed of more than a hundred individual components and in two different forms: liquid oil and solid powder. Most of the oil formed plant extracts are water-insoluble and often called essential oils. Plant extracts may be extracted from plants through steam distillation, maceration, cold pressing, and solvent extraction or may be synthesized (Kerrola, 1995). Plant extracts are of potential interest due to their potential biological functions, such as antiviral, antimicrobial, antioxidant, and anti-inflammatory effects (Baydar et al., 2004, Sökmen et al., 2004, Dundar et al., 2008, Liu et al., 2012; 2013a, b; 2014a, b). This may lead to the ability to use plant extracts to replace antibiotics in-feed to improve performance and health of animals (Pettigrew, 2006; Stein and Kil, 2006). Based on the literature, plant extracts may improve animal health through several mechanisms such as direct suppression of the proliferation of pathogens, alteration of gut microbial populations, and enhancement of immune functions. Lee et al. (2004), Calsamiglia et al. (2007), and Bakkali et al. (2008) have well reviewed plant extracts and their biological effects.

Anti-microbial activities of various plant extracts have been well organized. Plant extracts exhibit a wide spectrum of antibacterial activities against gram-negative and gram-positive bacteria, including *Escherichia*, *Salmonella*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *Clostridium*, and *Mycobacterium* (Hammer et al., 1999; Dorman and Deans, 2000; Wong et al., 2008). Considering the large number of different groups of chemical components present in plant extracts, it is not surprising that several modes of action are



involved in their anti-microbial effects. Plant extracts may directly kill pathogens due to their hydrophobicity and due to the high percentage of phenolic compounds (Farag et al., 1989; Dorman and Deans, 2000; Lambert et al., 2001; Carson et al., 2002; Burt, 2004; Xu et al., 2008). Certain bioactive components in plant extracts may prevent the development of virulent structures in bacteria (Burt et al., 2007). The active components in plant extracts may also disturb the enzyme system of bacteria and then block the virulence of the microbe (Ankri and Mirelman, 1999).

The anti-inflammatory effects of plant extracts have been well identified in *in vitro* cell culture models. Plant extracts (i.e. carvacrol, cinnamaldehyde, eugenol, etc.) inhibit the production of pro-inflammatory cytokines and chemokines from endotoxin stimulated immune cells and epithelial cells (Lang et al., 2004; Lee et al., 2005; Tung et al., 2008; Liu et al., 2012). The research on the potential mechanisms of anti-inflammatory effects of plant extracts are still limited. It has been indicated that anti-inflammatory activities may be partially mediated by blocking the NF- $\kappa$ B activation pathway (Jobin et al., 1999; Lee et al., 2005; Choi et al., 2007).

Plant extracts are also proposed to be used as antioxidants in animal feed, which will protect animals from oxidative damage caused by free radicals. The antioxidative properties of extracts of oregano, thyme, clove, pepper, lavender, and basil have been evaluated by many studies *in vitro* (Economou et al., 1991; Gülçin et al., 2004; Oboh et al., 2007). Slamenova et al. (2008) indicated that carvacrol given in drinking water reduced the level of DNA lesions induced in freshly isolated hepatocytes and testicular cells by H<sub>2</sub>O<sub>2</sub>, which could be associated with an increase of antioxidant activity of liver and testicular cells in these animals. Frankić et al. (2010) demonstrated that supplementation of plant extracts to pigs reduced the DNA damage in lymphocytes, which indicated their potentially beneficial effects on the immune system under dietary-induced oxidative stress. The antioxidant activity of plant extracts is highly correlated with their chemical compositions (Teissedre and Waterhouse, 2000). The presence of phenolic OH groups in thymol, carvacrol, and other plant extracts act as hydrogen donors to the peroxy radicals produced during the first step in lipid oxidation, thus retarding the hydroxyl peroxide formation (Farag et al., 1989; Djeridane et al., 2006).

### 7.1. Plant extracts on performance and health

Use of phytogetic products as feed additives for swine has been reviewed by Windisch et al. (2008). Allan and Bilkei (2005) reported that sows fed diets containing 1,000 mg/kg oregano (dried leaf and flower of *Origanum vulgare*, consisted of 50% cold-pressed essential oil of *O. vulgare*) had lower annual sow mortality rate, lower sow culling rate, increased farrowing rate, increased number of liveborn piglets per litter, and decreased stillbirth rate. However, in a different study, no beneficial effect was observed from sows fed with oregano EO (Ariza-Nieto et al., 2011).

A large variation was observed in growth performance of newly-weaned pigs fed diets supplemented with different types of plant extracts. Supplementation of 1,000 mg/kg of oregano extract increased weight gain but reduced disease incidence of weaned pigs (Sads and Bilkei, 2003), however, no benefits on performance of weaned pigs were observed in other studies (Manzanilla et al., 2004; Neill et al., 2006; Nofrarias et al., 2006).

Although failing to find beneficial effect on productive performance, feeding plant extracts have been indicated to improve gut health by modulating gut microbiota or immunity. Manzanilla et al. (2004) and Nofrarias et al. (2006) reported that a mixture of plant extracts (oregano, cinnamon and Mexican pepper) increased stomach contents and percentage of DM, suggesting an increased gastric retention time. In addition, those mixture decreased ileal

total microbial mass and increased the Lactobacilli to Enterobacteria ratio. Michiels et al. (2010) also indicated that supplementing with 500 mg/kg carvacrol and thymol reduced the number of intra-epithelial lymphocytes and increased villus height/crypt depth in the distal small intestine. Disease challenge study with a pathogenic *E. coli* indicated that inclusion of low dose (10 mg/kg) of capsicum oleoresin, turmeric oleoresin, or garlicon reduced frequency of diarrhea and improved gut health of weanling pigs, as indicated by improved intestinal villi height and gut barrier function and integrity (Liu et al., 2013b). Feeding plant extracts also reduced the systemic and gut inflammation of weanling caused by *E. coli* infection (Liu et al., 2013b). It has also been demonstrated that inclusion of capsicum oleoresin, garlicon, and turmeric oleoresin in diets for weanling pigs challenged with porcine reproductive and respiratory syndrome virus (PRRSV) may help alleviate negative impacts of infection, as indicated by reducing viral load and serum concentrations of inflammatory mediators, and shortening the time of fever in PRRSV-infected pigs (Liu et al., 2013a). Thus, there is strong evidence that inclusion of several different plant extracts may contribute to improved health of weanling pigs.

In the grower-finisher period, the application of different levels and different sources of plant extracts also shows benefits on growth performance. Cullen et al. (2005) and Janz et al. (2007) reported pigs fed with a garlic-treated diet had higher ADG, ADFI, and feed conversion ratio compared with the pigs fed with the control diets. Grela et al. (1998) observed a significant improvement in ADG and feed conversion ratio with the use of an herb mixture (great nettle, garlic, wheat grass) in the diet of pigs from 25 to 105 kg. Dunshea et al. (2003) demonstrated an improvement in growth performance with the inclusion of vanillylnonamide, a capsaicin analog, in the diets of finisher pigs.

In summary, plant extracts are strong candidates to replace antibiotics in feed to improve growth performance and health of pigs in different stages. However, the potential benefits of plant extracts may differ due to the large variation in the composition of plant extracts, resulting in difficulty in comparing the efficiency of different plant extracts. The major bioactive compounds of plant extracts are polyphenols, and their composition and concentration vary according to the plant, parts of the plant, geographical origin, harvesting season, environmental factors, storage conditions, and processing techniques. The predominant constituents in the popular plant extracts include anethol, capsaicin, carvacrol, cinnamaldehyde, curcumin, eugenol, thymol, and others. The chemical compositions of many plant extracts were summarized by Burt (2004) and Surburg and Panten (2006) and plant extracts may contain 20 to 60 components in different concentrations. The major components can constitute up to 85% of the plant extracts, whereas other components are present only as trace amounts. For example, the concentration of thymol from the same species of plants, *Origanum vulgare* can vary from trace amounts to 64%, while *Thymus vulgaris* may vary from 10% to 64%. Another component, carvacrol, has been reported to range from trace amounts to 80% in the extract of *O. vulgare* and from 2% to 11% in *T. vulgaris* (Lawrence and Reynolds, 1984; Burt, 2004). Thus, the variations in active compounds in plants and plant-derived products have to be considered when plant extracts are used as potential alternatives to antibiotic growth promoters. Nevertheless, one commercial blend of plant extracts (containing carvacrol, cinnamaldehyde and capsicum oleoresin) has been approved in the European Union as the first botanical feed additive for improving growth performance in broiler chickens and livestock. Synthetic components have been used by the industry to keep the finished products more consistent and reduce the cost. In addition, different encapsulation methods by using polymer particles, liposomes, and solid lipid nanoparticles have also been adopted to control the release of plant extracts and

effectively deliver them to the middle of the small intestine, which is believed to most effectively improve intestinal health (Sherry et al., 2013; El Asbahani et al., 2015). However, more research will be needed to evaluate the efficacy of plant-derived products and the modes of action in order to improve the utilization of plant extracts on animal health and production.

## 8. Conclusions

Several feed additives may be effective in regulating intestinal environments and improving pig growth performance if diets without antibiotic growth promoters are fed. For example, acidifiers may modulate GI tract pH, and thereby affect the diversity of the gut microbiota and increase nutrient digestibility. Zinc and copper have beneficial effects on gut health beyond their nutritional values, which is likely due to the antibacterial effects of these minerals. Probiotics and DFM may increase the population of beneficial microbes in the GI tract by serving as substrates for specific microbes in the gut or by directly adding beneficial microbes in the gut. Nucleotides and plant extracts also affect the gut microbiota and may boost immunity of pigs. Thus, there are a number of feed additives that potentially may be used in diets fed to pigs, but the main challenge with all of these additives is the fact that results obtained so far have been inconsistent. The reason for this inconsistency may be that efficiencies of each additive are diet dependent and also dependent on the health status of the animals. It is, therefore, not possible to recommend a specific additive that will have positive effects in all diets, but it is likely that if no antibiotic growth promoters are used, at least some additives will be beneficial in diets fed to pigs.

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