

Non-antibiotic feed additives in diets for pigs

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ABSTRACT

A number of feed additives are marketed to assist in boosting the pigs' immune system and reduce the negative impact of weaning and other environmental challenges. The most commonly used feed additives include acidifiers, zinc and copper, pre-biotics, 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 demonstrated to result in improved pig performance or improved immune function of pigs. It is also possible that use of pre-biotics, 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.

Introduction

Producing pigs without using antibiotic growth promoters represents a challenge. Disease problems often are elevated and general performance is compromised on farms practicing non-medicated swine production. That is true in particular during the immediate post-weaning period whereas antibiotics can often be removed from diets fed to growing-finishing swine without introducing major disease problems (Wierup, 2001). Because of the difficulties associated with producing pigs without antibiotics, many producers are looking for alternative growth promoters or management strategies, but at this point, no "magic bullets" are available. Producing swine without in-feed antibiotics requires a combination

of different strategies. These strategies can be divided into three categories: Management strategies, nutritional strategies, and alternative dietary supplements. Many different approaches have been proposed within each strategy, but the one thing these approaches have in common is that they all aim at improving the pigs' ability to prevent pathogenic bacteria from colonizing in the intestinal system. This can be accomplished via an improved immunological response to pathogens or via mechanisms that prevent the pathogens from adhering to intestinal tissue, and thus, reduce the damaging effects of the pathogens. In the present contribution, some of the additive that may be used to ameliorate the negative impacts of removing antibiotic growth promoters from the diets will be discussed.

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 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 the dietary acidifiers has not been fully elucidated, but the following mechanisms have

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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).

Organic acids

The most commonly used organic acids include formic acid, fumaric acid, and citric acids and supplementation of diets with organic acids usually result in a reduction in stomach pH (Eidelsburger et al. 1992b; Roth et al. 1992a; Radcliffe et al. 1998). 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 on 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., 2014). 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).

Recently, combinations of organic acids and medium-chain fatty acids have been demonstrated to have stronger antibacterial 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 effect on the digestibility of nutrient as well as growth performance. Moreover, Kuang et al. (2015) also reported the positive effects on the digestibility of nutrients and on growth performance (Upadhaya et al., 2014; 2016).

Inorganic acids

Inorganic acids commonly used in diets include hydrochloric acid, sulfuric acid, and phosphoric acid and 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 reported (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).

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 have been 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⁺-K⁺-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).

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 *E. coli* counts, but did not improve growth performance of pigs (Ahmed et al., 2014). Upadhaya et al. (2014; 2016) 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., 2016).

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.

Minerals

Minerals are inorganic elements needed by pigs for maintenance, growth, and reproduction. Minerals needed in quantities greater than 100 mg per 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 also have antimicrobial

properties and they are therefore often added to diets in quantities greater than what is needed to fulfil the nutritional requirements.

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 80-100 ppm 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). It has also been reported that high levels of Zn stimulates 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 than ZnO (Ward et al., 1996) and addition of 250 ppm of Zn-methionine to nursery diets have beneficial effects that are equivalent to addition of 2,000 ppm 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, 2015). Supplementation of high levels of Zn leads to deeper crypts and greater thickness of the duodenum (Carlson 1998), 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, 2003).

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 5-6 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., 1928; 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 CuSO₄ in enhancing growth rate and feed efficiency in pigs (Cromwell et al., 1998). Use of TBCC instead of CuSO₄ 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 CuSO₄, 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 alter the 3-dimensional structure of bacterial proteins, which prevents bacteria from performing their normal functions (Thurman et al., 1989). 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).

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., 2011a). 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).

Prebiotics

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., 2016). 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 short-chain fatty acids (SCFA) and a reduced concentration of pathogenic bacteria (Smiricky-Tjardes et al., 2003).

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

Beneficial effects of prebiotics in diets fed to pigs have been related to increased fermentability, because the subsequent synthesis of short chain fatty acids results in reduced intestinal pH. Increased concentrations of short chained fatty acids 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, 2016). 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).

Results of several studies have also demonstrated that the prebiotic lactulose may result in greater concentrations of IgM and IgA and improved immunity against *Salmonella typhimurium* (Naquid et al., 2015). Likewise, increased cell mediated immune response, IL-1 β gene expression, and serum levels of IL-1 β , 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 -(1-4) linkages) and galacto-mannan oligosaccharides (Yin, et al., 2010). Although it has been hypothesized that increased concentrations of lactic acid in the small intestine as a result of dietary prebiotics may be the reason for the positive impact on the immune system, the exact mechanism is still unknown (Naquid et al., 2015). In contrast, supplementation of 0.1% of chicory, mannan oligosaccharides, or 0.02% of chitosan had no

effect on growth performance or serum concentration of IgA (Li et al., 2016). There are many factors that may affect growth performance and immune responses to prebiotic supplementation of diets fed to pigs, which may be the reason for the inconsistent results that have been observed (Patterson, 2005).

Direct fed microbials

Direct-fed microbials (DFM), 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 (Kremer, 2006). Direct-fed microbials are categorized into three main groups: Bacillus, lactic acid-producing bacteria, and yeast (NRC, 2012). 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 (Nielsen et al., 2005; de Lange et al., 2010). However, after weaning of pigs, populations of lactic-acid bacteria diminish; therefore, supplementation of weaned pig diets with lactic-acid producing DFM may be beneficial (Stein and Kil, 2006). Yeast cultures may produce enzymes and vitamins along with other nutrients, which have been reported to produce a variety of responses when fed to swine (Kornegay et al., 1995).

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 performance (Kenny et al., 2011; Cromwell, 2013). Furthermore, DFM may

reduce immune stimulation, indicated by a reduction in proinflammatory cytokines in enterocytes, which may shift energy normally used for excessive immune stimulation to growth, thereby improving feed efficiency (Cho et al., 2011). The use of DFM in swine diets is expected to increase due to the recent restrictions on the use of antibiotic growth promoters. Continued use of fibrous co-products also may increase the use of DFM because it has been suggested that combining DFM and prebiotics (i.e., symbiotics) may increase the efficacy of DFM (de Lange et al., 2010).

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, 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. The DFM continue to survive due to their ability to produce enzymes that degrade the feed and produce short chained fatty acids as a by-product of fermentation. The short chained fatty acids produced are utilized by the pig as an energy source, and the increased short chained fatty acids 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 (Nielsen et al., 2013). 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).

Efficacy of Direct-Fed Microbials

Published reviews have concluded that the efficacy

of DFM added to swine diets is inconclusive because variable results have been observed (Pollmann, 1986; 1992; Nosiainen and Setälä, 1993; Stavric and Kornegay, 1995). However, a recent review has stated that results of 30 out of 31 nursery pig trials indicated an increased ADG and gain to feed ratio (G:F) due to DFM supplementation (Kremer, 2006). Therefore, reports prior to 2000 may not be appropriate today because the development of DFM and the technology associated with DFM has improved, which may lead to the increased efficacy.

Addition of 0 , 5.0×10^4 , 6.7×10^6 , or 7.5×10^8 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). Furthermore, 2 different diets were fed; a diet based on egg protein and a diet based on soybean protein and each diet affected pig metabolism as one would expect, but DFM supplementation interacted with each diet in their effects on weanling pig metabolism and immunity, indicating that the effects of DFM are most likely diet dependent (Merrifield et al., 2013; Bailey, 2016).

Kornegay et al. (1995) investigated the ability of a yeast culture containing *Saccharomyces cerevisiae* to increase nutrient digestibility by pigs because yeast culture supplementation increased cellulolytic bacteria in the rumen of cows (Dawson et al., 1990) and was suggested 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

Bacillus subtilis, *Bacillus licheniformis*, and *Bacillus pumilus*.

A more recent study utilizing 270 wean-to-finish pigs tested the dose of DFM (0 , 0.64×10^6 , 1.28×10^6 , 1.92×10^6 viable spores of BioPlus 2B, which contained *Bacillus licheniformis* and *Bacillus subtilis* in a 1:1 ratio; Chr. Hansen, Hørsholm, Denmark) and duration of DFM addition (weaning only or wean-to-finish) to diets, and 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 *Bacillus 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). Lee et al. (2014) concluded that the observed improvements were mostly caused by producing the *Bacillus subtilis* DFM using solid substrate fermentation (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 *Bacillus licheniformis* and one strain of *Bacillus 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 *Bacillus 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 *Lactobacillus 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 *Lactobacillus 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/MT *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 short chained fatty acid 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). These results are supportive of the *Bacillus* DFM and fiber degradation hypothesis.

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 DFM appear to be more beneficial for weaning 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.

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., 2011a; Jiang et al., 2015). Substantial evidence, however, for

many of these claims has yet to be reported.

Composition of yeast cells

In the search for alternatives for antibiotic growth promoters, compounds that can augment natural immunity and inhibit the growth of pathogenic bacteria have been increasingly researched and for these reasons, yeast cells are being targeted as potential substitutes for antibiotic growth promoters. 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 β -D-glucans and α -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.

Morphological effects

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 de Peet-Schwering et al., 2007).

Immune responses and diarrhea

Many of the beneficial effects of yeast products are claimed to be the result of immunopotentiality (Kogan and Kocher, 2007; Molist et al., 2014) and there is evidence that yeast and yeast products stimulate the immune system because the β -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). 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 α -D-mannans in yeast have possible antioxidant effects (Kogan and Kocher, 2007). In particular, the α -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). However, results of other studies were less affirmative (Weedman et al., 2011).

Growth performance and nutrient digestibility

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 immunopotentiating 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 summary, 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.

Nucleotides

Nucleotides are ubiquitous molecules with considerable structural diversity. They are composed of a nitrogenous base linked to a pentose sugar to which at least one phosphate group is attached. The pentose sugar may be a ribose for a ribonucleic acid (RNA) or a 2'-deoxyribose for a deoxyribonucleic acid (DNA). The nitrogenous base can be a purine or a pyrimidine. Pyrimidine bases are composed of six membered rings and comprise uridine, cytosine, and thymine. Purine bases have an additional five membered ring and comprise adenine, guanine, and hypoxanthine. 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). When the phosphate group is absent, the compound is known as a nucleoside. 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.

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 intraepithelium 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- α and IL-6 in blood serum 2 and 4 hours after an *E. coli* injection (Hung, 2015), and increase plasma and serum concentrations of IgA (Lee et al., 2007; Sauer et al., 2012) 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 *Cl. 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., 2012).

Dietary nucleosides 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 Youping, 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). 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., 2012; 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., 2011b; Hung, 2015; Waititu, 2016).

Plant extracts

Plant extracts are secondary plant metabolites and can be obtained naturally from plant materials. They are in two different forms, liquid oil or solid powder. Most of the oil formed plant extracts are water-insoluble and often called essential oils. Plant extracts have been largely applied for human nutrition and improvement of human health 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). Various plant extracts exhibit a wide spectrum of antibacterial activities against gram-negative and gram-positive bacteria (Hammer et al., 1999, Dorman and Deans, 2000, Wong et al., 2008) with several different modes of action. First, plant extracts may directly kill pathogens due to their hydrophobicity, which enables them to partition into the lipids of the bacterial cell membrane and mitochondria, resulting in leakage of critical intracellular materials (Carson et al., 2002, Burt, 2004, Xu et al., 2008). Second, plant extracts contain high percentage of phenolic compounds, which possess strong antibacterial properties (Frag et al., 1989, Dorman and Deans, 2000, Lambert et al., 2001). Third, the active components in plant extracts may disturb the enzyme

system of bacteria and then block the virulence of the microbe (Ankri and Mirelman, 1999). Fourth, certain bioactive components in plant extracts may prevent the development of virulent structures in bacteria, such as flagella that is critical for bacterial adhesion (Burt et al., 2007).

The anti-inflammatory effects of plant extracts have been widely reported with in vitro cell culture models. Essential oils from clove, tea, garlic, cinnamon and others have potential anti-inflammatory activities because they may suppress the production of TNF- α , IL-1 β , and nitric oxide from lipopolysaccharide (LPS)-induced mouse macrophages (Lang et al., 2004, Lee et al., 2005, Tung et al., 2008). Lang et al. (2004) reported that garlic extract also may inhibit intestinal epithelial cell secretion of several chemokines, such as IL-8, IP-10, and MIG, which mediate the inflammatory response by recruitment of various circulating leukocytes into the inflamed tissue. In vitro anti-inflammatory activities of several plant extracts (Carvacrol, capsicum oleoresin, cinnamaldehyde, garlic, eugenol, anethol, and turmeric oleoresin) with porcine alveolar macrophages have also been demonstrated and plant extracts suppressed the production of pro-inflammatory cytokines (TNF- α and IL-1 β) from LPS-stimulated macrophages (Liu et al., 2012), which indicate that these plant extracts may have anti-inflammatory effects. The modes of action for the anti-inflammatory activities of plant extracts are not clear, but effects may be partially mediated by blocking the NF- κ B activation pathway (Jobin et al., 1999, Lee et al., 2005, Choi et al., 2007). For example, curcumin may block cytokine-induced NF- κ B DNA binding activity, RelA nuclear translocation, I κ B α degradation, I κ B serine 32 phosphorylation, and I κ B kinase activity (Jobin et al., 1999).

Effects of dietary plant extracts on weaning pig health

Newly weaned pigs are subjected to a combination of stressors that predispose them to diarrhea, which may adversely impact survival (Moeser and Blikslager, 2007). Manzanilla et al. (2004) and Nofrarias et al. (2006) suggested that plant extracts may improve gut health. They reported that a mixture of plant extracts standardized to 5% (wt/wt) carvacrol, 3% cinnamaldehyde, and 2% capsicum oleoresin (oregano, cinnamon and Mexican pepper), increased stomach contents and percentage of dry matter suggesting an increased gastric retention time. In addition, the plant extracts decreased ileal total microbial mass and

increased the lactobacilli:enterobacteria ratio. Michiels et al. (2010) also indicated that supplementing diets 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.

Escherichia coli post-weaning diarrhea often results in economic losses due to mortality, morbidity, decreased growth performance, and cost of medication (Fairbrother et al., 2005, Nagy and Fekete, 2005). Enterotoxigenic E. coli are the most dominant types of pathogenic E. coli that cause diarrhea in both pre-weaning and post-weaning piglets (Hampson, 1994, Nagy and Fekete, 1999). In recent research, three plant extracts (capsicum oleoresin, garlison, and turmeric oleoresin) were tested with an in vivo pathogenic E. coli challenge to determine the effects of individual plant extracts on diarrhea and gut health of weaning pigs (Liu et al., 2013b). Results indicated that supplementation with individual plant extracts reduced overall frequency of diarrhea of pigs. Supplementation of plant extracts also improved ileal villus height and upregulated the mRNA expression of the mucin 2 (MUC-2) gene, which indicated that the reduced diarrhea score was likely due to improved gut barrier function and integrity (Liu et al., 2013b). Pigs infected with E. coli had increased number of white blood cells, serum pro-inflammatory cytokines (TNF- α) and acute phase protein (haptoglobin) and increased recruitment of macrophages and neutrophils in the ileum. Dietary supplementation with plant extracts reduced white blood cells, neutrophils, serum TNF α and haptoglobin and the number of macrophages and neutrophils in the ileum compared with the control diet. These observations indicate feeding low dose of plant extracts reduced both systemic and local inflammation caused by E. coli infection. Microarray results indicated that feeding plant extracts enhances the integrity of membranes, especially several tight junction proteins. Supplementation of plant extracts downregulated the expression of genes related to antigen processing and presentation and other immune response-related pathways, indicating that these plant extracts may attenuate the immune responses caused by E. coli infection (Liu et al., 2014). It has also been demonstrated that inclusion of capsicum oleoresin, garlison, and turmeric oleoresin in diets for weaning pigs challenged with porcine respiratory disease 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.

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