



Review Article

Intestinal challenge with enterotoxigenic *Escherichia coli* in pigs, and nutritional intervention to prevent postweaning diarrhea

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ABSTRACT

Gut health of nursery pigs immediately after weaning is tightly associated with their growth performance and economic values. Postweaning diarrhea (PWD) is one of the major concerns related to gut health of nursery pigs which often is caused by infections of enterotoxigenic *Escherichia coli* (ETEC), mainly including F4 (K88)⁺ and F18⁺ *E. coli*. The main virulence factors of ETEC are adhesins (fimbriae or pili) and enterotoxins. The common types of fimbriae on ETEC from PWD pigs are F18⁺ and F4⁺. Typically, PWD in pigs is associated with both F18⁺ and F4⁺ ETEC infections whereas pre-weaning diarrhea in pigs is associated with F4⁺ ETEC infection. Enterotoxins including heat-labile enterotoxins (LT) and heat-stable peptide toxins (ST) are associated with causing diarrhea in pigs. At least 10⁹ to 10¹⁰ ETEC are required to induce diarrhea in nursery pigs typically lasting 1 to 5 days after ETEC infection. Antibiotics used to be the most effective way to prevent PWD, however, with the increased bacterial resistance to antibiotics, alternatives to the use of antibiotics are urgently needed to prevent PWD. Immunoprophylaxis and nutritional intervention of antimicrobial minerals (such as zinc oxide and copper sulfate), organic acids, functional feedstuffs (such as blood plasma and egg yolk antibodies), direct fed microbials, phytobiotics, and bacteriophage can potentially prevent PWD associated with ETEC. Some other feed additives such as nucleotides, feed enzymes, prebiotic oligosaccharides, and clay minerals can enhance intestinal health and thus indirectly help with preventing PWD. Numerous papers show that nutritional intervention using selected feed additives can effectively prevent PWD.

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1. Introduction

The gut is the main portion of the digestive tract and also the largest portion of the immune system in animals. The gut is responsible for digestion of feed, absorption of nutrients, and protection of the body from toxins and pathogens. Maintaining the gut in a good health condition, especially in nursery pigs, is an important basis of successful pig production.

Pathogenic infection is one of the major challenges impairing gut health in nursery pigs. The first part of this review focuses on pathogenesis of enterotoxigenic *Escherichia coli* (ETEC) impairing gut health because of its significant impacts on global swine production. *E. coli* postweaning diarrhea (PWD), also named as postweaning enteric colibacillosis, is a crucial factor causing mortality of nursery pigs in the global swine production. The infection of ETEC in nursery pigs may induce diarrhea during the first 1 or 2 weeks of postweaning periods usually resulting in dehydration, reduced weight gain, and death (Verdonck et al., 2007). The severity of PWD can be further contributed by various factors, such as weaning stress, dietary changes, and deficiency of milk antibodies (Fairbrother et al., 2005).

Diarrhea in pigs occurs frequently due to infections of single or multiple types of *E. coli*: ETEC, vero- or shiga-like toxin producing *E. coli*, necrotoxicogenic *E. coli*, enteropathogenic *E. coli*, enterohaemorrhagic *E. coli*, enteroaggregative *E. coli*, and enteroinvasive *E. coli*. Among these, ETEC is the most prevalent cause of severe and

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watery diarrhea in nursing and nursery pigs (Nagy and Fekete, 2005).

Recently, the incidence of *E. coli* infection became a more frequent reason of sudden death or severe diarrhea in the global swine production. Postweaning diarrhea is usually related with F4 (K88)⁺ and F18⁺ *E. coli* infections (Zhang et al., 2007). The *E. coli* isolates are often found to be resistance to a wide range of antimicrobials including spectinomycin, apramycin, trimethoprim–sulfonamide, and neomycin (Amezcuca et al., 2002; Lanz et al., 2003; Maynard et al., 2003). The prophylactic use of antibiotics largely contributed to antimicrobial resistance, and the frequency and range of antimicrobial resistance were seen among ETEC strains (Choi et al., 2002; Docic et al., 2003; Maynard et al., 2004). Additionally, the antimicrobial growth promoters (AGP) such as avoparcin, bacitracin, spiramycin, and tylosin with prophylactic activity have been forbidden in the EU, Korea, and USA, and potentially followed by other countries. In fact, removal of AGP in feed brought an increased incidence of diarrhea, weight loss, and mortality mainly caused by the presence of *E. coli* in nursery pigs (Casewell et al., 2003). However, a long-term practice of AGP removal in feed would eventually help the gut health in pigs by reducing antibiotic resistance of ETEC strains (Maynard et al., 2004). With the increasing incidence of *E. coli*-associated diarrhea and resistance to antibiotics by ETEC strains, it is important and urgent to develop some alternatives to the use of conventional AGP. The second part of this review focuses on alternative nutritional strategies to prevent ETEC infection and PWD in pigs.

2. Virulence factors of *E. coli*

The main pathotype of *E. coli* causing PWD in pigs is ETEC. These bacteria adhere to the epithelium of the small intestine. Even though ETEC do not directly induce detrimental morphological changes, they secrete enterotoxins impairing enterocyte functions by increasing fluidity and reducing water absorption. Virulence factors refer to molecules produced by microorganisms which cause interactions with the host. The main virulence factors of ETEC are adhesins with hair-like appendages (fimbriae or pili) (Proft and Baker, 2009) and enterotoxins (peptides or proteins). Receptors expressed by the host are important for pathogenesis by adhesins and enterotoxins. The species specificity of a receptor makes ETEC strains highly specific to the type of a host.

2.1. Fimbriae correlation to adhesins

The first step of a pathogenic process is the interaction between adhesins and ligands on microvilli of the small intestine, which is an essential step for bacterial attachments to microvilli without morphological destruction. Fimbriae are the most prevalent type of adhesive surface antigens of ETEC. The common types of fimbriae found on ETEC from PWD pigs are F18 and F4 (Frydendahl, 2002). Typically, PWD in pigs are shown to be associated with both F18 and F4 fimbriae whereas pre-weaning diarrhea in pigs is shown to be primarily associated with F4 fimbriae (Fairbrother et al., 2005).

2.2. F18 fimbriae

Fimbriae are long and thin appendages with proteins protruding 0.5 to 1.5 μm from the surface of a bacterium. There are typically 100 to 300 fimbriae peritrichously distributed on the surface of a bacterium (Ottow, 1975; Klemm, 1985; Van de Broeck et al., 1999a). Fimbriae can be morphologically classified into 2 categories: pili and fibrillae (Simons et al., 1994). Pili have rigid structures (7 to 8 nm diameter and an axial hole), whereas fibrillae are relatively thin and flexible with undefined diameter. The F18 fimbriae,

belonging to fibrillae, are 1- to 2-mm long filaments based on a major structural protein called FedA (15.1 kDa) with a zigzag pattern around the helical axis (Hahn et al., 2001). The F18 fimbriae occur as 2 antigenic variants, F18ab and F18ac, where the “a” is a common antigenic factor, and “b”, “c” are specific factors (Sarrazin and Bertschinger, 1996). Before 1995, F18 fimbriae were designated as F107 (which is now recognized as F18ab), 2134P, or 8813 (which is now recognized as F18ac) (Imberechts et al., 1992, 1994; Rippinger et al., 1995). The F18ab are poorly expressed *in vitro* and usually found on Shiga toxin-producing *E. coli* (STEC) and ETEC, whereas F18ac are easier to be expressed *in vitro* and usually found on ETEC (Wittig et al., 1995; Nagy et al., 1997). The F18⁺ ETEC strains often produce heat-stable enterotoxins including STa and STb, whereas heat-labile enterotoxin (LT) is infrequently produced (Rippinger et al., 1995; Francis, 2002). Colonization of ETEC in the small intestine is promoted by a fimbriae–receptor interaction. The F18 fimbriae bind to glycoproteins on microvilli of the small intestine (Nagy and Fekete, 2005). Compared with F18ac fimbriae, F18ab fimbriae only have 9 to 12 different amino acids (Imberechts et al., 1994). Depending on genetic backgrounds, some pigs lack a receptor for F18 fimbriae and thus those pigs are resistant to colonization of F18⁺ ETEC. Occurrence of F18⁺ ETEC infections can be increased from 6% of pigs with the F18-resistant genotype to 87% in genetically susceptible pigs (Frydendahl et al., 2003).

2.3. F4 (K88) fimbriae

The F4 fimbriae are proteins with a long filamentous polymeric surface. Simons et al. (1994) also showed that the structure of F4 fimbriae can be varied from a thin, flexible, and extended structure to a wider, rigid, and condensed structure. The F4 fimbriae occur as 3 antigenic variants (F4ab, F4ac, and F4ad). The “a” is a common antigenic factor and “b”, “c”, and “d” are specific factors. These 3 F4 variants have slight different amino acid compositions on their major subunit (FaeG) (Mooi and de Graaf, 1979). The major subunits of F4ab and F4ad are composed of 264 amino acid residues whereas the major subunit of F4ac has 262 amino acid residues. Among the 3 F4 variants, the F4ac is more popular (Guinée and Jansen, 1979). Researchers examined 44 F4⁺ ETEC isolates from PWD pigs and found that 96% carried the F4ac fimbriae genes and 4% carried the F4ab fimbriae genes (Choi and Chae, 1999). Another study found that 98% of F4⁺ ETEC from 237 PWD pigs carried F4ac variant (Alexa et al., 2001). The expression of the fimbrial genes is usually affected by environmental factors including temperature, pH, and culture medium (De Graaf and Mooi, 1986). Compared with the F18 fimbriae, FaeG is both the major fimbrial subunit and the adhesion for F4 fimbriae.

All F4 adhesins bind carbohydrates of glycoproteins on the intestinal epithelial cells and intestinal mucus (Erickson et al., 1994; Grange et al., 2002). Meanwhile, F4ad adhesins preferably bind to glycolipids, whereas F4ab and F4ac adhesins are more likely to bind glycoproteins (Grange et al., 1999).

2.4. Other adhesins

Based on fimbriae types, F5(K99), F6(987P), F17(Fy/Att25), and F41 are found in both pigs and calves (Table 1). These fimbriae usually associated with ETEC diarrhea in nursing pigs, and these fimbriae are found individually or together with F18 or F4 on ETEC from PWD pigs. Kwon et al. (2002) found that genes for F5, F6, and F41 were present at 4%, 10%, and 2%, respectively, of ETEC from PWD pigs. Since the reduced lactation period and weaning age over years, it is common to see that these fimbriae are detected on ETEC from PWD pigs (Fairbrother et al., 2005).

Table 1
Fimbrial and afimbrial adhesins of enterotoxigenic *E. coli* (ETEC) in animals.¹

Adhesin	Diameter, nm	Subunit size, kDa	Mannose sensitivity	Associated O-type	Receptor
F4 (K88) ab, ac, ad	2.1	27.6	MR ²	O8, O141, O149	Sialo-glycoprotein
F5 (K99)	5	16.5	MR	O8, O20, O101	Glycolipid (NeuGC-GM3)
F6 (987P)	7	17.2	MR-NH ²	O9, O20	Glycoprotein/lipid (sulfatide)
F17 (Fy)	3.4	20	MR	O101	Glycoprotein
F18ab, ac	3.5	17	MR-NH	O139, O141, O147, O157	Glycoprotein
F41	3.2	29	MR	O101	Glycoprotein
F43		32	MR		Glycoprotein
F165	4 to 6	17.5, 19	MR	O115	Glycoprotein
AIDA ²	NA	100	NA	O26	Glycoprotein

AIDA = adhesin-involved-in-diffuse-adherence.

¹ Adopted from Nagy and Fekete (2005).

² AIDA is an afimbrial adhesion.

3. Enterotoxins

Enterotoxins are extracellular proteins or peptides secreted from bacteria including ETEC. They affect the intestinal epithelium. Enterotoxins can be categorized to LT possessing large-molecular-weights (88 kDa) and heat-stable peptide toxins (ST) possessing small-molecular-weight (1 to 5 kDa) (O'Brien and Holmes, 1996).

3.1. Heat-labile enterotoxins

The *E. coli* LT consists of an A:B₅ protein structure. A subunit A is a single enzyme which is non-covalently associated with a subunit B that is a pentamer. A pentamer subunit B binds the toxin to its receptor (De Haan and Hirst, 2004). The 2 subunits A and B contain 240 and 103 amino acids, respectively (Nagy and Fekete, 2005). The subunit A consists of 2 fragments, A1 (ADP-ribosyl transferase) and A2 (peptide), and they are connected with a disulfide bond passing through a pore of the B subunit (Sixma et al., 1991; van Beers-Schreurs et al., 1992). Subunits A and B are originally synthesized in the cytoplasm, and processed to structure holotoxin in the periplasm. The subunit B binds predominantly to the monosialotetrahexosylganglioside (GM1) receptor on the cell surface (O'Brien and Holmes, 1996). After this step, the fragment of A1 will be translocated to the endoplasmic reticulum, and activate the adenylate cyclase system to increase cellular cyclic adenosine monophosphate (cAMP) inducing increased fluid and electrolyte secretion and decreasing their absorption (Fujinaga et al., 2003).

In addition to the function of enterotoxicity, LT may also act as an adhesion for binding of the bacteria to GM1 on the intestinal epithelial cell surface (Horstman et al., 2004). Berberov et al. (2004) showed that the elimination of LT genes reduced severity of diarrhea and colonization of ETEC on the small intestine of gnotobiotic pigs, which supports that LT may act as an adhesin.

3.2. Heat-stable enterotoxins

Heat-stable enterotoxins include STa and STb. The first discovered variant of ST is STa, which is a low molecular weight peptide composed of 18 amino acids with 3 disulfide bonds. The STa is soluble in water and methanol and then digested by proteolytic enzymes. It resists boiling water for 15 min and can be inactivated by agents that destroy disulfide bonds. The STb is a peptide composed of 48 amino acids with 4 cysteine residues involved in 2 disulfide bonds. The STb is not soluble in methanol but can be digested by proteolytic enzymes. The STa appears to be specifically associated with ETEC causing disease in neonatal animals, but may also cause PWD in pigs along with some other enterotoxins. The STb is closely related to diarrhea in pigs (Dubreuil, 1997).

4. Serogroups and serotypes of ETEC in postweaning diarrhea

With greater variations among toxin-encoding genes than among fimbriae genes, specific serogroups of ETEC are established to identify particular virulence genes. The ETEC serogroups include O and H serogroups which are determined based on lipopolysaccharide and flagella, respectively (Table 2). The O antigens are composed of repeated subunits that extend from the surface of bacteria (Wolf, 1997). Major serogroup of *E. coli* causing PWD in pigs is O149 (Orskov et al., 1969). Other serogroups that are frequently causing PWD in pigs are O8, O138, and O141 (Sojka, 1965; Salajka et al., 1992; Nagy et al., 1997; Nagy and Fekete, 2005; Francis, 2002; Frydendahl, 2002). The H serogroups are determined by the flagellar antigens, and serve as useful antigenic markers and as potential components of an ETEC vaccine. Only a few H serogroups are related to ETEC compared with O serogroups, and some H serogroups were strongly associated with an O serogroup such as O27:H7, O8:H9, and O148:H28 (Wolf, 1997).

5. Pathogenesis of *E. coli*

The PWD caused by *E. coli* is associated with an enormous proliferation of *E. coli*, and then their colonization in the small intestine through bacterial attachment to receptors on the small intestinal epithelium or in the mucus coating the epithelium. The proliferation process is necessary because 10⁹ to 10¹⁰ ETEC are experimentally required to induce diarrhea (Acres, 1985). Degree of colonization would determine if an infection can develop to cause disease conditions. Fimbriae attach to a specific receptor on the cell membrane of intestinal epithelial cells and also non-specifically in the mucus coating the intestinal epithelium. The ETEC that bear fimbriae F5, F6, and F41 mostly colonize in the distal jejunum and ileum, whereas F4⁺ ETEC has the ability to colonize in the entire jejunum and ileum. The F4⁺ adhesion occurs mostly in pigs and is mediated by F4 fimbriae.

The F4 receptors are fully expressed from birth to maturity in pigs (Fairbrother et al., 2005), whereas the F18 receptor would not be fully expressed until about 20 days of age in pigs (Nagy et al., 1992). Therefore, *E. coli* with F18 fimbriae do not cause disease symptoms in neonatal pigs. Although F18⁺ ETEC causes diarrhea in nursery pigs, it is shown that 11-week-old pigs had symptoms of diarrhea caused by F18⁺ ETEC. Some pigs do not have receptors for the F4 adhesin on intestinal epithelial cells which make them genetically resistant to infection by F4⁺ ETEC (Sellwood et al., 1975).

Antibodies (immunoglobulins) obtained from colostrums and milk protect nursing pigs from proliferation of *E. coli* strains in their intestine. Upon weaning, however, nursery pigs appear to be more susceptible to *E. coli* enteric infections (Deprez et al., 1986;

Table 2
O serogroups with associated fimbrial antigens and H antigens.¹

O serogroup	Associated fimbrial antigens	Associated H antigens	Comments
8	F4ab(K88ab), F4ac(K88ac)	H19	Less common than in earlier years
138	F18, F4ac ²	H–, H4	Sometimes Stx2e+
139	F18	H19	Most commonly associated with Edema disease
141	F18, F4ab, F4ac	H4	Sometimes Stx2e+
147	F4ac, F18	H6, H19	Sometimes Stx2e+
149	F4ac, F18 ²	H10, H19, H43, H–	Occasionally Stx2e+
157	F4ac	H19, H43	Occasionally Stx2e+

¹ Adopted from Fairbrother et al. (2005).

² This occurs infrequently.

Sarmiento et al., 1988). A wide range of viruses infect the porcine intestine and this may alter the intestinal environment facilitating bacterial infection. Lecce et al. (1982) showed that infections of both rotavirus and ETEC strain resulted in severe diarrhea than individual infection supporting that viral damage of the epithelium could enhance the colonization of *E. coli*.

In general, pigs with ETEC can show watery diarrhea which lasts about 1 to 5 days after ETEC infection. Some pigs have shown sudden death without diarrheal symptoms but with intestinal edema. Diarrhea usually results in a significant dehydration, either due to a failure of the intestine to reabsorb or absorb fluid or due to a great increase in fluid secreted into the intestine. The basic mechanism by LT and ST enterotoxins is to impair function of the small intestinal epithelial cells, resulting in increased secretion of water and electrolytes (Na⁺ and Cl[–]), decreased fluid absorption, and increased dehydration and acidosis.

Structure and the mode of action of LT enterotoxin are well understood and similar to *Vibrio cholera* (CT). As mentioned before, the B subunits of LT bind mainly to the GM1 receptor on the cell surfaces. Immediately after the adhesion of enterotoxin to a cell surface, a fragment of A domain (A1) will translocate into a cell and activate the adenylate cyclase system increasing cAMP level. In turn, protein kinase A is stimulated by cAMP phosphorylating the cystic fibrosis transmembrane conductance regulator (CFTR), and then causing Cl[–] secretion from the luminal surface of enterocytes (Thiagarajah and Verkman, 2003; de Haan and Hist, 2004).

The STa is shown to stimulate the guanylate cyclase system inducing intracellular accumulation of cyclic guanosine monophosphate (cGMP), impairing absorption of water and electrolytes (Na⁺ coupled Cl[–]) on villus tips, and finally resulting in an elevated secretion of water and Cl[–] from crypt cells (Forte et al., 1992). However, the molecular mechanism of how STb causes disease symptoms is not well understood. Dubreuil (1997) showed that STb binds to sulfatide (3-O-sulfogalactosylceramide) of the intestinal epithelial cells and then internalized to the cells. Whipp et al. (1981) found that STb, however, does not increase cyclic nucleotide levels in the epithelial cells but enhance the secretion of non-chloride anions from intestinal epithelial cells.

6. Applications of preventing postweaning diarrhea associated with ETEC

6.1. Diagnosis of ETEC infections

Diagnosis of ETEC infection symptoms should include the detection of virulence factors such as adhesins and enterotoxins. These can be detected by traditional *in vitro* tests including slide latex agglutination test and ELISA (Thorns et al., 1989). Adhesive fimbriae are efficiently detected *in vivo* under an immunofluorescent method using absorbed monoclonal or polyclonal anti-fimbrial antibodies (Isaacson et al., 1978). Compared with

fimbriae, enterotoxins are much harder to detect *in vivo* with traditional methods which make it necessary to develop some advanced molecular level techniques. The DNA-based molecular detection methods (PCR and DNA hybridization) are generally used to detect known virulence factors (Franck et al., 1998). Additionally, real-time PCR is well used to detect ETEC (Fukushima et al., 2003). However, the traditional ETEC detection methods are still required prior to measuring gene expression and discovering new virulence factors (Czirok et al., 1992; Thorns et al., 1989).

6.2. Immunoprophylaxis

The newborn pigs have a capacity to establish immune reactions such as tolerance or defense against mucosal antigens (Rothkotter et al., 2002). Active intestinal mucosal immunization is needed to protect newly weaned pigs due to their lack of passive lactogenic immunity. The vaccines for PWD should be able to activate the mucosal immune system and antigen-specific immunoglobulins (A and M) responses to induce a protective mucosal immunity (Bianchi et al., 1996; Van den Broeck et al., 1999b).

Three types of vaccines for ETEC have been experimentally applied to pigs. The first is intramuscular injectable vaccines to stimulate systemic immunity increasing circulating antibodies to keep intestinal bacteria levels low enough to be non-pathogenic (Van den Broeck et al., 1999b). Intramuscular injection of F4 fimbriae induced a systemic immunoglobulin A (IgA) response and enhanced the reduction of excretion of F4⁺ *E. coli* (Van der Stede et al., 2003).

The second is an oral administration of live attenuated or live wild-type non-enterotoxigenic *E. coli* strains carrying the fimbrial adhesins to pigs. Immunization can be done orally to nursing pigs and via drinking water to nursery pigs at least 1 week before the expected onset of diarrhea. This can stimulate the intestinal colonization by these *E. coli* which induces the secretion of intestinal antibodies, and finally blocks the adherence of ETEC. A wide range of on-farm studies have shown a reduced mortality and reduced use of antibiotics after oral administration of a live attenuated or live non-enterotoxigenic F4⁺ *E. coli* strain to pigs immediately after weaning (Fuentes et al., 2004; Ruan and Zhang, 2013; Fairbrother et al., 2016).

The third is the oral administration of purified fimbriae, instead of the whole bacteria, to pigs. The use of such vaccine results in a specific mucosal immune response in the intestines and may cause a significant decrease in fecal excretion of the pathogenic *E. coli*. Previous studies demonstrate that oral immunization of nursery pigs with purified F4 fimbriae induces an F4-specific systemic and mucosal immune responses protecting pigs from a subsequent F4⁺ ETEC challenge (Van de Broeck et al., 1999b; Verdonck et al., 2004). Purified fimbriae can be orally delivered to the small intestine via enteric-coated pellet even though interaction of purified fimbriae to coating polymer reduced biological activity of purified fimbriae

needing further research to find effective way of delivering purified fimbriae to the small intestine without losing biological activity (Huyghebaert et al., 2005; Srivastava et al., 2016).

6.3. Antimicrobial mineral

Zinc is shown to have important roles in maintaining the gut epithelial barrier integrity and function (Vallee and Falchuk, 1993). Numerous studies demonstrated that therapeutic level of zinc oxide (ZnO) improved the growth performance of nursery pigs (Hahn and Baker, 1993; Hollis et al., 2005). Roselli et al. (2003) showed that ZnO reduced damages to cellular membranes in enterocytes induced by F4⁺ ETEC infection. Nusrat et al. (2000) indicated that the mechanism of ZnO enhancing growth of nursery pigs is not only due to its antibacterial activity but also due to enhancing gut epithelial tight junction function and structure by modulating inflammatory cytokines.

Copper is shown to act as a growth stimulant and potential inhibitor of bacterial infection in pigs (Adewole et al., 2016). Direct contact of copper to bacterial cells can cause oxidative damages to bacterial cell membrane and DNA by causing production of reactive oxygen species (Mathews et al., 2015). Copper is also shown to be the cause of contact killing of bacteria by damaging bacterial membrane, increasing cellular copper, and finally degrading bacterial DNA (Grass et al., 2011). Robert et al. (2012) found that copper causes oxidative damage of bacterial membrane, subsequent loss of membrane integrity, and finally death of *E. coli*. Bactericidal effects of copper were obtained from copper alloy as well as copper sulfate (CuSO₄).

CuSO₄ is widely used as a growth-promoting feed additive with its antimicrobial activities. Nursery pigs fed a diet containing 170 mg/kg CuSO₄ showed a significant decrease in fecal coliform and *E. coli* (Hojberg et al., 2005).

6.4. Acidifiers

Hydrochloric acid secreted from the stomach can act as an antibacterial chemical and subsequently contributes to the gut barrier integrity against pathogens. However, at weaning, intake of solid feed increases pH of the gastrointestinal tract. Thus, dietary supplementation of organic acids could be an effective way to control pH of the gastrointestinal tract and growth of bacteria in the stomach and intestine.

Dietary supplementation of lactic acid or citric acid was effective to prevent PWD and thus enhance growth of nursery pigs (Tsiloyiannis et al., 2001). Bosi et al. (2007) showed that dietary supplementation of formic acid reduced fecal excretion of total *E. coli*, whereas enhanced intestinal morphology and growth performance of nursery pigs. An *in vitro* study showed that supplementation of formic acid or multiple acids to liquid feed reduced Enterobacteriaceae counts (Canibe et al., 2007). During an outbreak of PWD, dietary supplementation of various organic acids could be effective in reducing the occurrence and severity of diarrhea and the presence of F4⁺ ETEC in the intestine (Tsiloyiannis et al., 2001).

6.5. Blood plasma

Feeding a diet with blood plasma to nursery pigs has shown an inhibitory effect on intestinal colonization of ETEC. Owusu-Asiedu et al. (2002) showed improved weight gain and reduced frequency of ETEC-associated diarrhea in early weaned pigs fed a diet with blood plasma which could partly be due to the presence of specific anti-ETEC antibodies in blood plasma. Bosi et al. (2004) found that a non-mediated diet with blood plasma enhanced growth performance, protected F4-receptor positive pigs against ETEC infection, and reduced the ETEC-induced inflammatory response in pigs. Diets

supplemented with non-immune plasma powder had significant effects on reducing fecal excretion of F18⁺ *E. coli* and clinical symptoms when piglets were infected with F18⁺ *E. coli* (Nollet et al., 1999). Studies indicate that high level plasma Ig content may be responsible to prevent pathogenic infection helping to maintain the gut barrier function and the integrity of small intestine (Hernández et al., 2010; Weaver et al., 2014a; Hedegaard et al., 2016).

6.6. Egg yolk antibodies

The egg yolk antibodies obtained from laying hens immunized with specific bacterial fimbrial antigens is a relatively economic source of antibodies with the ability to protect against ETEC infection in pigs (Marquardt et al., 1999). To determine the mechanism of egg yolk antibodies (EYA) in preventing ETEC infection, researchers monitored the binding of F4⁺ ETEC to the small intestine of piglets provided with EYA. The results showed reduced adhesion of F4⁺ ETEC to small intestinal mucosal epithelium when EYA were provided to pigs. This indicates that EYA can block the binding of ETEC fimbriae to the mucosal receptor (Jin et al., 1998) if EYA is provided to nursery pigs before ETEC infection. Kim et al. (1999) showed that dietary supplementation of EYA can be an effective means to reduce PWD in nursery pigs. Marquardt et al. (1999) demonstrated that nursery pigs fed EYA from chickens immunized with purified F4 showed reduced diarrhea and mortality following F4⁺ ETEC challenges. Kiarie et al. (2009) found that pigs fed EYA had increased villus height to crypt depth ratio and reduced ileal pH following an oral challenge of F4⁺ ETEC.

On the other hand, some studies observed no difference in the incidence of diarrhea or mortality when pigs were fed EYA (Friendship, 2002; Chernysheva et al., 2003). Further studies are needed to validate the role of EYA prior to a practical application in feeding nursery pigs.

6.7. Direct fed microbials (DFM)

The direct fed microbials are defined “preparations containing living microorganisms that positively influence the colonization and composition of gut microflora and have a stimulating effect on the digestive processes and the immunity of the host” (Fuller, 1992). Roselli et al. (2005) reviewed that a certain DFM, such as *Sterptococcus faecium*, *Bifidobacterium lactis*, *Bacillus toyoi*, is effective in reducing intestinal infection of pathogenic ETEC and intestinal inflammatory responses. Zhang et al. (2010) indicated that *Lactobacillus rhamnosus* GG (LGG) was effective in ameliorating diarrhea in post-weaning piglets induced by F4⁺ ETEC, possibly by modulating intestinal microflora, enhancing intestinal antibody defense, and regulating production of systemic inflammatory cytokines. Diets supplemented with *Bacillus toyoi* or *B. licheniformis* reduced the incidence and severity of diarrhea, as well as the number of ETEC in the intestine (Kyriakis et al., 1999). Jin and Zhao (2000) found that *Enterococcus faecium* inhibited the adhesion of F4⁺ ETEC to the intestinal mucosal layer where receptors for adhesive fimbriae of F4⁺ ETEC are present.

However, the effectiveness of DFM could be dependent on strains or condition of pigs. Some other studies showed that certain DFM were ineffective when individually added to the feed (Broom et al., 2005; Taras et al., 2006). Sanders and Huis in't Veld (1999) proposed that multi-strains and multi-species DFM to be more effective than mono-strain DFM due to the specific health effects based on genera, species, and strain. Huang et al. (2004) indicated that the complex *Lactobacilli* preparation improved pigs' performance for 2 weeks after weaning, enhanced resistance to *E. coli* infection in the GI tract.

Our recent study (Loftus, 2015) compared efficacy of mono-strain DFM with multi-strain DFM. Our mono-strain DFM based on *Lactobacillus acidophilus* did not affect growth performance and loin quality of growing-finishing pigs, whereas the dietary inclusion of multi-strain DFM based on *L. acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, and *E. faecium* enhanced weight gain and improved carcass yield without affecting loin quality. Another recent study done in our lab (Sun, 2013) demonstrated that the supplementation of multi-strain DFM based on *L. acidophilus*, *L. casei*, *B. thermophilum*, and *E. faecium* enhanced growth performance of pigs orally challenged with F18⁺ ETEC. The relevant occurrence of F18⁺ ETEC was reduced in the mucosal epithelium of the small intestine in nursery pigs challenged with F18⁺ ETEC when pigs were fed a diet supplemented with multi-strain DFM.

6.8. Phytobiotics

Phytobiotics, also known as phytogetic feed additives, are plant-derived products supplied to the feed in order to improve growth performance of pigs by enhancing intestinal health. Phytobiotics comprise a wide variety of herbs, spices, and products derived thereof, and are mainly essential oils (Windisch et al., 2008). After the complete ban on antibiotic growth promoters in EU, phytobiotics became attractive alternatives in animal diets. Various studies have been conducted to find out the roles of plant extracts and essential oils in growth promoting, antimicrobial, antioxidative, and other properties (Kommera et al., 2006; Li et al., 2012; Maenner et al., 2014; Zeng et al., 2015).

Increased weight gain and decreased diarrhea occurrence were observed in nursery and growing pigs by using the extracts of *Macleaya cordata* (Wild) R. Br. as feed additives at the concentration ranging from 15 to 50 mg/kg (Kantas et al., 2015; Liu et al., 2016). Tataru et al. (2008) showed improved weight gain of nursery pigs supplied with allicin as the functional component in aged garlic extract. Devi et al. (2015) showed that improved weight gain and apparent total tract digestibility of ash in pigs by feeding phytogetic additive combination (clove, cinnamon, and fenugreek) after dietary challenge with F4⁺ ETEC. Dietary supplementation of blend extracts of cinnamon, thyme, and oregano inhibited colonization of pathogenic *E. coli* in the intestine of nursery pigs (Namkung et al., 2004). Research conducted by Newton et al. (2002) and Liu et al. (2016) verified that sanguinarine acts as an antimicrobial agent by promoting the growth of beneficial bacteria in the GIT of pigs and inhibiting the colonization of pathogenic bacteria. When supplied with blend plant extracts (oregano, cinnamon, and Mexican pepper), nursery pigs had decreased total microbial mass and increased *Lactobacilli* to enterobacteria ratio in the ileum (Manzanilla et al., 2004). Khan et al. (2009) showed that pathogenic strains of *E. coli* were sensitive to the following plant extracts: *Acacia nilotica*, *Syzygium aromaticum*, and *Cinnamomum zeylanicum*. The most common constituents against oxidative reaction were volatile oils from mint, especially products from rosemary. The antioxidant capacity of many phytogetic compounds may be inferred to contribute to protection of feed lipids from oxidative damage (Windisch et al., 2008). Unlike the other non-antibiotic growth promoters, which were already well studied, phytobiotics were relatively new types of feed additives. Further researches on phytobiotics are needed to focus on their modes of action and aspects of their application.

6.9. Bacteriophage

Bacteriophages are a group of viruses infecting bacteria and kill those infected bacteria with high species-specificity. Bacteriophages have been applied widely and therapeutically in pig

production as alternatives to antibiotic use against PWD caused by ETEC (Zhang et al., 2015). Specific bacteriophage (PP01) was isolated from feces of pigs which were resistant to the infection of *E. coli* O157:H7, and its high concentration in the feces of these pigs might indicate its capability of suppressing ETEC in the host (Morita et al., 2002). Lee et al. (2016) showed dietary supplementation of bacteriophages to nursery pigs with PWD caused by F4⁺ ETEC reduced *E. coli* adhesion in the ileum and cecum and increased villus height to crypt depth in the duodenum and jejunum.

6.10. Nucleotides

Nucleotides act as bioactive molecules that play a role in metabolic, structural, and regulatory functions (Sauer et al., 2012) as well as immune system maintenance and re-dox balance (Salobir et al., 2005). Typical nursery diets do not include sufficient amount of nucleotide (Martinez-Puig et al., 2007). Dietary supplementation of nucleotides in nursery pig diets have shown positive effects on growth performance, intestinal hyperemia, and immunity stimulation (Sauer et al., 2012; Superchi et al., 2012; Weaver and Kim, 2014; Waititu et al., 2016). Li et al. (2015) showed that dietary supplementation of nucleotides fed to nursery pigs orally challenged with F4⁺ ETEC enhanced growth performance, nutrient digestibility, immune status, microbial balance, and reduced diarrhea (Li et al., 2015).

6.11. Other potential supplements

Some other studies recorded significant improvement in growth performance, immunity, and intestine morphology and the decrease in the frequency, severity and duration of diarrhea in weaned pigs fed diets supplemented with feed enzymes, prebiotic oligosaccharides, clay minerals, and milk coproducts (Weaver et al., 2013, 2014b; Passos et al., 2015; Tactacan et al., 2016; De Greeff et al., 2016).

7. Conclusion

Gut health of nursery pigs immediately after weaning is tightly associated with their growth performance and economic values. Post-weaning diarrhea is one of the major concerns related to gut health of nursery pigs and is often caused by infection of *Enterotoxigenic E. coli*. Antibiotics used to be the most effective way to prevent PWD, however, with the increased bacterial resistance to the antibiotics, alternatives to antibiotics are urgently needed. Immunoprophylaxis and nutritional application of antimicrobial minerals (such as zinc oxide), organic acids, functional feedstuffs (such as blood plasma and egg yolk antibodies), direct fed microbials, and bacteriophage can potentially prevent PWD associated with ETEC. Some other feed additives such as nucleotides, feed enzymes, prebiotic oligosaccharides, and clay minerals can enhance intestinal health and indirectly help with preventing PWD. This review introduced research evidence from numerous papers indicating that nutritional intervention using selected feed additives can effectively prevent PWD.

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