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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 10^9 to 10^{10} 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. Immunopropylaxis 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.

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*, necrotoxigenic *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 (Amezcua 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, spiromycin, 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).

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