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Chapter Dietary Intervention to Reduce E. coli Infectious Diarrhea in Young Pigs

Peng Ji, Xunde Li and Yanhong Liu

Abstract

Postweaning piglets are immediately imposed to remarkable environmental and psychosocial stressors, which adversely affect their intestinal development and health and predispose them to diarrhea. The ratio of postweaning mortality is 6–10% and may rise up to 20% with poor management strategies. Diarrhea per se accounts for 20–30% of cases of mortality in weanling pigs. E. coli postweaning diarrhea is one of the most important causes of postweaning diarrhea in pigs. This diarrhea is responsible for huge economic losses due to high mortality and morbidity, weight loss, and cost of medication. Burgeoning evidence suggested feed-based intervention are one of the promising measures to prevent postweaning diarrhea and to enhance overall health of weaned pigs. Although the exact protective mechanisms may vary and are still not completely understood, a number of feed ingredients or feed additives are marketed to assist in boosting intestinal immunity and regulating gut microbiota. The promising results have been demonstrated in several nutrients (i.e., functional amino acids, organic acids, micro minerals, nondigestible carbohydrates, and antimicrobial peptides), non-nutrients (i.e., phytochemicals and probiotics), and many other feed additives. The efficiencies of each candidate may differ based on their exact modes of action, the basal diet formulation, and the health status of pigs.

Keywords: dietary intervention, E. coli infectious diarrhea, ETEC, young pigs

1. Introduction

Escherichia coli (E. coli), a Gram-negative rod-shaped bacterium, was first discovered in 1885 by Theodor Escherich, who noted that E. coli are highly prevalent in the intestinal microflora of healthy individuals and have potential to cause disease when directly inoculated into extraintestinal sites. Diarrheagenic E. coli can be further divided into six groups: enterotoxigenic E. coli (ETEC), enteropathogenic E. coli, enterohemorrhagic E. coli, enteroinvasive E. coli, diffusely adhering E. coli, and enteroaggregative E. coli [1]. Different groups of diarrheagenic E. coli express different virulence genes, exhibit different adhesion characteristics, and therefore have different mechanisms of pathogenicity. This book chapter only covers the infection caused by ETEC.

ETEC is the major etiological agent causing acute watery diarrhea in post-weaning piglets. The duration of diarrheal symptom may be shortened by

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Antibiotic treatment, but ETEC is relative refractory to common antibiotics. A growing evidence suggested some nutritional components (e.g., functional amino acids, nondigestible carbohydrates, etc.) and non-nutrients (e.g., phytochemicals, probiotics, etc.) may provide preventive benefits to control ETEC infection. In general, the compounds listed above are supplemented into animal feed with small amount, also named as feed additives. The exact protective mechanisms are largely unknown and may differ for each compound. However, based on the literature review, these feed additives may alleviate ETEC infection by targeting at least one of the following mechanisms:

1. Modification of intestinal microbiota by directly killing pathogens or competitively inhibiting the binding of pathogens and toxins to gut epithelium.
2. Regulation or stimulation of host immunity that may include intestinal mucosal immunity and systemic immune defense.

2. E. coli infectious diarrhea

E. coli postweaning diarrhea is an important cause of death in weaned pigs. This diarrhea is responsible for economic losses due to mortality, morbidity, decreased growth performance, and cost of medication [2, 3]. ETEC are the most predominant types of pathogenic E. coli that cause diarrhea in both preweaning and postweaning piglets [4, 5].

2.1 Clinical signs

Clinical signs of ETEC infection in pigs include reduced appetite, depression, weakness, rapid dehydration, watery diarrhea (light orange-colored feces), anorexia, and shock due to hypovolemia and electrolyte imbalance [6, 7]. Cyanotic discoloration may appear on the tip of the nose, the ears, and the abdomen. The rectal temperature of infected pigs is generally normal. Pigs may spontaneously recover within 1 week if the infection is mild. However, severe infection may cause death within 12 hours, even without the symptoms of diarrhea. Dehydration of the carcass and distension of the small intestine by colorless mucoid fluid are most common necropsy characteristics for ETEC-infected pigs. During ETEC infection, bacteria normally line the epithelial cells of the intestine rather than invade the mucosa; gross and histological lesions, therefore, may not be directly caused by the bacteria. However, physiological changes in the intestine may be caused by the toxins released by ETEC [8, 9].

2.2 The pathogenesis of E. coli infection

There are two major virulence factors involved in the pathogenesis of ETEC infection, including the expression of fimbriae that enable the attachment of bacteria to the small intestinal epithelial cells, and the production of toxins by the colonized ETEC [10–12]. In addition, other structural components from E. coli, such as capsular polysaccharides, cell wall lipopolysaccharides (LPS), and iron-binding proteins, may also be involved in the pathogenesis of ETEC [13]. The endotoxins produced by ETEC could induce intestinal physiological changes, which are leading to the disrupted water and fluid absorption and ion secretion, finally causing dehydration and acidosis. The bacterial structural components could also initial a cascade of immune stimulation, resulting in intestinal inflammation and systemic inflammation [14, 15].
Fimbriae of *E. coli*

Fimbriae are proteinaceous appendages located at the outer membrane of the bacterial cells. They are straight or kinky shapes. The major role of fimbriae is to facilitate the adhesion and colonization of ETEC at the small intestinal mucosa [16–18]. The adhesion of bacteria is extremely important for ETEC infection. It will stabilize the location of bacteria in the intestinal lumen, which allow the pathogens with better access to luminal nutrition, facilitate the secretion and delivery of endotoxins through epithelium, and help the bacteria penetrate into the tissue if needed [16–18]. Diarrheagenic ETEC may express many kinds of fimbria, including F4 (K88), F5 (K99), F6 (987p), F18, etc.; F4 (K88) and F18 ETEC are the most common pathogenic ETEC in young pigs.

F4 fimbriae are typically identified in ETEC isolated from pre- and postweaning pigs. F4 ETEC tend to colonize throughout the whole segments of the small intestine in pigs [19]. F4 fimbriae are encoded by the *fae* operon, which comprises genes coding for several regulatory proteins, distal tip protein, minor subunits, and a major subunit, FaeG, that enable F4+ ETEC binding to specific receptors on intestinal brush border cells [2, 3, 20]. There are three naturally occurring serological variants of F4 fimbriae, including F4ab, F4ac, and F4ad. They are interchangeable by changing a residue stretch in the FaeG protein. However, F4ac variant is the most common F4 fimbriae variant expressed in porcine pathogenic ETEC in the United States [2, 3]. The adhesion receptors of F4 fimbriae appear to be glycoconjugates, including glycoproteins and glycolipids, which have been identified from the brush borders of epithelial cells, intestinal membranes, and mucosa [21, 22]. It is interesting to note that F4ad adhesin appears to preferentially bind to glycolipids, whereas F4ab and F4ac adhesins preferentially bind to glycoproteins [22–24].

F18 fimbriae are associated with *E. coli* strains isolated from postweaning diarrhea and edema disease in pigs. These fimbriae are long flexible appendages that show a characteristic zigzag pattern [16]. Based on morphological, serological, functional, and genetic characteristics, two antigenic variants of F18 fimbriae were determined and designated: F18ab and F18ac [25]. F18ab-positive strains are usually isolated from cases of edema disease, whereas F18ac-positive strains are associated with cases of postweaning diarrhea [16, 26]. F18 fimbriae are composed of protein subunits (FedA) with molecular weights of approximately 15.1 kDa [27]. Five structural genes (*fed A*, *fed B*, *fed C*, *fed E*, and *fed F*) encoded on a plasmid have been identified [28]. Among these genes, *fed E* and *fed F* genes are essential for F18 adhesion and fimbrial length [29]. However, receptors for F18 fimbriae actually increase with age and have not been detected in newborn pigs [30]. This may in part explain the reason why ETEC strains carrying F18 are more prevalent in weaned pigs.

Toxin effects

After adhering to the small intestinal surface, ETEC induce enteric infectious disease and diarrhea through release of enterotoxins, which stimulate copious secretion by the small intestinal mucosa. The enterotoxins include heat-labile toxin, heat-stable toxin, LPS, and Shiga toxins.

Heat-labile toxins.

Heat-labile toxin mainly accumulates in the periplasmic space, with limited amount appears on the surface of the bacteria. Heat-labile toxin consists of a single A subunit and five B subunits. The binding of B subunits to the monosialotetrahexosylganglioside (GM1) ganglioside on the cell surface facilitates the translocation of a fragment of A domain into the cell, which then activates the adenylate cyclase system and increases the expression of cyclic adenosine...
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DOI: http://dx.doi.org/10.5772/intechopen.91219

Surface, LPS is recognized by TLR4-MD-2 complex, which transduces intracellular LPS signals via several signal pathways [45], ultimately resulting in the activation of NFκB and subsequently the release of inflammatory cytokines [15].

2.2.2.3 Shiga toxin

Some ETEC strains that cause postweaning diarrhea possess additional genes that encode Shiga toxin, allowing them to cause edema disease as well [4]. Similar to heat-labile toxin, Shiga toxin is also a protein toxin, consisting of one A subunit and five B subunits. However, Shiga toxin has completely different mechanisms infecting cells, in comparison with heat-labile toxins. Briefly, Shiga toxin first binds to the cells that possess the glycolipid receptors, globotriaosylceramide (Gb)3 or Gb4 [46, 47]. After binding, Shiga toxin is transported to the Golgi apparatus through endocytosis. The Golgi apparatus further transports Shiga toxin to the endoplasmic reticulum, where the subunit A is cleaved by trypsin and is separated into A1 and A2 subunits. The A1 subunit is released into the cytosol and subsequently impacts ribosomes [14]. Shiga toxin can inhibit protein synthesis and induce synthesis of cytokines, including IL-1, IL-6, IL-8, and TNF-α [47, 48]. In addition, Shiga toxin also induces DNA degradation and release of the cellular contents that facilitate proteolytic attack on neighboring cells, contribute to cell apoptosis, and have a toxic effect in the whole organism [14].

3. Dietary intervention on E. coli infectious diarrhea

Nutrients are compounds in feed ingredients that are essential to animal maintenance and production, by providing animals with energy, the building components for repair and growth, and the substances to regulate biological processes. Nutrients are generally grouped into six major classes: water, carbohydrates, proteins, lipids, minerals, and vitamins. With the exception of carbohydrates, all five classes of nutrients are indispensable and have to be provided through animal feed. In addition, a group of specific nutrients, such as functional amino acids, nondigestible carbohydrates, short-chain fatty acids (SCFA), and several micro minerals, has beneficial effects on animal health and performance beyond their nutritional contributions. Recently, a novel concept, non-nutrients, is illuminated to describe a group of dietary compounds, which has no nutrient contribution to animals, but have physiological activities beyond the nutritional pyramid, formulation practices, and feeding methods that similarly alter physiological condition. Emerging evidence suggested that several non-nutrient feed additives (i.e., plant extracts, probiotics, enzymes, etc.) improved animal health through modulating microbial ecology in the digestive tract and/or enhancing immune responses of animals to enteric infections.

3.1 Functional amino acids

Amino acids are defined as organic substances that contain both amino and carboxyl groups. Amino acids are classified according to their molecular weights, chemical structures, the composition of nitrogen and sulfur, and physiological functions. The 20 common proteinogenic amino acids shared by all animal species are further categorized into indispensable, semi-dispensable (conditional essential), and dispensable amino acids, dependent on their dietary essentiality. Functional amino acids are defined with a group of amino acids that are...
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Traditionally classified as dispensable amino acids, but with extra biological functions [49]. The well-investigated functional amino acids are the arginine family, which includes arginine, glutamine, glutamate, aspartate, proline, etc. The basic functions of these amino acids have been well summarized by Wu et al. [49, 50], which include but not limited to (1) providing substrates for the synthesis of tissue protein; (2) impacting hormone synthesis and secretion; (3) regulating endothelial function, vasodilation, and blood flow; (4) affecting nutrient metabolism; and (5) maintaining acid-base balance and whole-body homeostasis.

Large amounts of literature have reported the impacts of dietary supplementation of functional amino acids on health and performance of newly weaned pigs. For example, supplementation of 0.2 to 1% L-arginine could enhance growth performance and alleviate the negative effects of different insults or challenges in young pigs [51–54]. Administration of proline was shown to improve mucosal proliferation, intestinal morphology, as well as intestinal tight junction of weaned pigs [55]. Dietary supplementation of glutamine or dipeptides that are composed of glutamine has shown positive impacts on intestinal integrity, enzyme activities, and growth performance of weaned pigs [56–58]. Several mechanisms are highly involved in the benefits of the arginine family on intestinal health of weaned pigs, which could prevent the intestinal dysfunction caused by *E. coli* infectious diarrhea in weaned pigs. First, these amino acids could provide major fuel for small intestinal epithelial cell proliferation and provide energy required for intestinal ATP-dependent metabolic processes [59]. Second, catabolism of these amino acids provides precursors or substrates for the synthesis of nitric oxide, polyamines, and creatine, which are important regulators in blood flow, intestinal integrity and secretion, and epithelial cell repair and migration [60–62]. Third, glutamine is also a major substrate for glutathione synthesis, which is an important endogenous antioxidant in cells regulating the homeostasis of free radicals [63]. Fourth, these amino acids could enhance intestinal secretory IgA production via regulating the intestinal microbiota and immunity [53, 64].

### 3.2 Fatty acids

Dietary fat and lipids are extremely important for animal health and production. They have three major fundamental roles in swine nutrition by providing energy, compound lipids, and steroids to animals. Triglycerides and free fatty acids are the primary forms of metabolic energy storage and transport in the animal body. Short-chain fatty acids and medium-chain fatty acids (MCFA) have recently attracted increased research attention as potential candidates to reduce enteric infectious disease in animal production due to their potential antimicrobial activities [65–67]. SCFA are fatty acids with a chain of less than six carbon atoms, which are primarily produced by hindgut fermentation of dietary fiber. The most abundant SCFA in the gastrointestinal tract are acetic (C2), propionic (C3), and butyric acid (C4). They are the major fuel source for colonocytes and are essential for maintaining the normal metabolism of colon mucosa, including colonocyte growth and proliferation [68, 69]. Butyric acid has received particular attention and has been widely investigated to enhance disease resistance of weaned pigs. Addition of this acid directly to a swine diet may be limited because of its pungent odor and unpalatable flavor. Thus, the salt form (sodium or calcium) or glyceryl form (monobutyrin or tributyrin) of butyric acid has been adopted in animal feed industry. One major advantage of glyceryl forms in comparison with salt forms is that they stay intact in the stomach and are slowly released as butyrate and/or monobutyrin in the small intestine where pancreatic lipase appears [70]. Many research have confirmed the positive protective effects of sodium butyrate or tributyrin on intestinal health of pigs.
Copper is also an essential component of several metalloenzymes including cytochrome oxidase and lysyl oxidase. Copper is highly involved in oxidation-reduction reactions, transport of oxygen and electrons, antioxidant system, and many other metabolic functions, including cellular respiration, tissue pigmentation, hemoglobin formation, and connective tissue development [82]. In general, neonatal pigs only require 5–6 mg/kg of Cu for normal metabolism [85, 99], and Cu requirement decreases as animal gets older. Cu deficiency may lead to critical dysfunctions and hypocuprosis in pigs [100]. Pigs may also suffer from microcytic anemia and bone abnormalities [101, 102]. Addition of pharmacological levels of Cu (125–500 mg/kg) in pig diets has been a common practice to reduce postweaning diarrhea and improve growth performance [103–106]. The beneficial effects of pharmacological Cu have been attributed to its bacteriostatic and bactericidal properties [107, 108]. Similar to zinc, many Cu forms could be used in animal feed, including copper sulfate (CuSO\(_4\)), copper chloride (CuCl\(_2\)), tribasic copper chloride (Cu\(_2\)(OH)\(_3\)Cl), and copper citrate. Copper sulfate and copper chloride are the most common supplementing forms. Tribasic copper chloride has been suggested to have similar bioavailability but less negative impacts on phosphorus digestibility and intestinal microbiota than copper sulfate [109–111]. Chelated Cu, such as Cu citrate, has greater availability than inorganic Cu sources, which may be used in animal feed as low dose, resulting in reduced Cu excretion [112].

3.4 Prebiotics and probiotics

Prebiotics are a category of nutritional compounds that may not share similar structures but have the ability to improve the growth of beneficial microorganism in the gastrointestinal tract. Gibson et al. [113] offered a definition of prebiotics, which contains three key aspects: resistance to digestion, fermentation by the large intestinal microbiota, and a selective effect on the microbiota associated with health-promoting effects. Most well-studied prebiotics are nondigestible oligosaccharides or polysaccharides [114]. For instance, inulin-type prebiotics are a group of nondigestible carbohydrates that mainly comprise fructose, including inulin, oligofructose, and fructo-oligosaccharides. They are commonly used in the pig industry and human foods [115]. Galactooligosaccharides that exist in human milk have been reported to have prebiotic effects by enhancing colonic health of breast-fed infants [116]. Many other naturally occurring prebiotics have been reported as well, including polydextrose, trans-galactooligosaccharides, xylo-oligosaccharides, lactulose, pyrodextrins, and isomalto-oligosaccharides. However, a few other nondigestible carbohydrates are not categorized as prebiotics (e.g., mannan-oligosaccharides, β-glucan etc.), but manifest health-promoting functions [117]. For example, a growing evidence demonstrates that β-glucans, either produced by bacteria or extracted from different sources (i.e., cereal, algae, and fungi), could boost host immunity, therefore enhancing disease resistance of human and animals [118–120].

Probiotics, also known as direct-fed microbials, are live microorganisms and, when administered in adequate amounts, confer a health benefit on the host [121]. Probiotics are categorized into three main groups, including Bacillus, lactic acid-producing bacteria, and yeast [122]. Based on the Food and Drug Administration instruction, the term probiotics is used for human microbial products, whereas the term direct-fed microbials is used for the US feed industry. However, “probiotics” are interchangeably used with human and animal feed worldwide. Bacillus-based probiotics are spore-forming, which makes them thermostable and able to survive at low pH. Bacillus-based probiotics have been identified as potent producers of extracellular fiber-degrading enzymes, which may aid nutrient digestion and utilization [123]. Lactic acid-producing bacteria are not spore-forming; therefore, their...
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survival during feed processing is of concern [124]. Lactic acid-producing bacteria dominate the gastrointestinal tract of the nursing pig [125], which helps reduce the pH in the gut by producing lactic acid through fermentation, inhibiting enteric pathogens [126], and improving host immunity [124, 127]. However, after weaning of pigs, the concentration of lactic acid-producing bacteria diminishes; therefore, supplementation of weaned pig diets with lactic acid-producing probiotics may be beneficial [122]. Yeast include a broad range of products that may be available in pig feed, including whole live yeast cells, heat-treated yeast cells, ground yeast cells, purified yeast cell cultures, and yeast extracts. The efficacy of yeast-based products varies depending on their forms. Yeast or yeast-based product supplementation may boost feed intake and overall growth performance, augment mucosal immunity, promote intestinal development, adsorb mycotoxins, reduce postweaning diarrhea, and modulate gut microbiota in weaned pigs [128–131].

The most notable effect of prebiotics and probiotics is their modification of intestinal microbiota. They may control or prevent pathogenic bacterial infection by specifically stimulating the growth of beneficial microorganisms in the intestine. The beneficial microorganisms may include but not limited to Bifidobacteria and Lactobacilli, which have confirmed benefit to suppress the growth of pathogenic microorganisms, such as E. coli, through the potential mechanisms described below. For example, the desired bacteria produce SCFA and lactic acid, which may indirectly and specifically kill or inhibit the growth of pathogens [132]. The production of acids may reduce the pH of the intestinal environment, which is unsupportive of the growth of several pathogens [133]. The desired bacteria may produce antimicrobial compounds such as bacteriocins or antibiotics [134]. The desired bacteria compete the available nutrients against pathogens [135].

Many research articles have been published on the impacts of prebiotics and probiotics on infectious diseases in young pigs. For instance, supplementation of 8% inulin reduce the incidence and severity of postweaning diarrhea, probably by increasing SCFA production in the cecum and proximal colon [136]. The addition of fructo-oligosaccharide prevented the mortality and morbidity of weaned pigs infected with K88 ETEC [137]. Supplementation of β-glucan originated from different sources (yeast or algae) could enhance the resistance of pigs against K88 or F18 ETEC infection [120, 138]. The α-ᴅ-mannans from yeast could 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 [128]. Indeed, pigs supplemented with live yeast or a yeast fermentation product had reduced disease-related stress, diarrhea scores, duration of diarrhea, and shedding of E. coli and enhanced intestinal integrity in pigs challenged with ETEC [139–141]. Supplementation of Bacillus subtilis also enhanced disease resistance and growth performance and reduced diarrhea of weaned pigs infected with F18 ETEC [142].

3.5 Phytochemicals

Phytochemicals are secondary plant metabolites that are either naturally obtained from plant materials or directly synthetized. Phytochemicals are used in solid powder form, as crude extracts, or as concentrated extracts. The extracts are further classified as essential oils or oleoresins based on the extraction methods. Essential oils are volatile lipophilic substances obtained by cold extraction or distillation, whereas oleoresins are derived by nonaqueous solvents [143]. A few examples of well-known phytochemicals are curcumin, flavonoids, phenolic acids, isoflavones, carotenoids, etc. The major bioactive compounds in phytochemicals are polyphenols, terpenoids, alkaloids, or sulfur-containing compounds.
However, the composition and concentration of bioactive compounds in different phytochemicals may vary a lot, completely depending on the types of plant, the parts of plant, geographical origins, growing conditions, harvesting seasons, processing techniques, and storage conditions [144]. The in vitro biological properties of many phytochemicals have been well investigated, including antimicrobial, antioxidant, anti-inflammatory, and antiviral effects [145–148]. Therefore, phytochemicals have been largely applied in food processing, cosmetics, and other areas related to human nutrition and health.

Various phytochemicals have been reported to exhibit a broad spectrum of antimicrobial activities against Gram-negative and Gram-positive bacteria [149–151]. The potential mechanisms of action of antimicrobial activities of phytochemicals are described below. Many phytochemicals are lipophilic, which could damage bacterial membrane, eventually causing the leakage of intracellular materials and cell death [152–156]. In addition, the phenolic compounds possess strong antibacterial properties by inhibiting virulence factors, such as enzymes and toxins [157–159]. Lastly, certain bioactive components may also prevent the development of virulent structure (i.e., flagella) in bacteria, therefore inhibiting ETEC adhesion and toxin binding [160, 161].

Our previously published research reported that dietary supplementation of 10 mg/kg of capsicum oleoresin, garlic botanical, or turmeric oleoresin reduced the frequency of diarrhea and enhanced disease resistance of pigs infected with F18 ETEC [162]. The active components in these phytochemicals are capsaicin, propyl thiosulfonates, and curcuminoides, respectively. The results of gene expression profiles in ileal mucosa indicated that supplementation of these phytochemicals modified the expression of genes related to mucin production, cell membrane integrity, and antigen processing and presentations in ETEC-infected pigs [163]. In addition to the enhanced intestinal mucosal health, pigs fed with those phytochemicals had less recruitment of macrophages and neutrophils in the ileum [162]. These observations also suggest that the weaned pigs supplemented with those phytochemicals actually had less gut inflammation than infected control. The gene expression profile analysis by microarray also confirmed the reduced gut inflammation by feeding those phytochemicals to weaned pigs [163]. The phytochemicals discussed above can be naturally obtained from seasonings that are commonly used in kitchen. Many other phytochemicals have been thoroughly investigated to against ETEC infection as well. For example, the anti-diarrheal activity of back or green tea extract has been revealed, because the reduced net fluid and electrolyte losses were observed when F4 ETEC-infected jejunal segments were perfused with black or green tea extract [164]. The administration of cranberry extract (1 g/L) in drinking water also reduced the diarrhea of F18 ETEC-infected piglets [165].

3.6 Antimicrobial peptides

Antimicrobial peptides, also called host defense peptides, are polypeptides that are naturally occurring molecules in various organisms from prokaryotes to mammals. Antimicrobial peptides can be synthesized as recombinant molecules, such as recombinant lactoferrin, or can be isolated from bacteria, insects, vertebrates, or plants, such as bovine lactoferrin and plant defensins [166, 167]. Most of antimicrobial peptides are cationic (positively charged) and amphiphilic (hydrophobic and hydrophilic). Antimicrobial peptides were firstly discovered in the 1980s. They can be classified into different groups based on the different amino acid components, structures, and biological function. The antimicrobial peptides derived from mammals are mainly classified into two families, defensins or cathelicidins. Defensins are further subgrouped into α-, β-, and θ-defensins according to the spacing...
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Feeding lysozyme-rich milk also tended to reduce fecal Enterobacteriaceae family, in which many prevalent enteric pathogens such as E. coli and Salmonella belong to. Similar results were also reported in one animal trial focusing on human lysozyme-rich milk. In this experiment, neonatal pigs were used and infected with F4 ETEC. Consumption of human lysozyme-rich milk (approximately 1300 mg/L lysozyme) increased survival rate, reduced diarrhea, and facilitated the recovery of infected pigs. Lysozyme treatment also increased the relative abundance of Lactobacillus in feces and enhanced intestinal integrity and mucosa immunity of these neonatal pigs.

4. Conclusions

Accumulating evidence has confirmed the importance of nutritional interventions, including modified feeding strategies and nutrient supplements, in the control of diarrheal diseases, and preventing enteric infection as the use of antibiotics will be progressively restricted in many countries. Interest is particularly growing in the use of probiotics and prebiotics as natural alternatives to antibiotics.

| Strain | Diet Intervention | Outcome | Reference |
|--------|-------------------|---------|-----------|
| K88    | Milk from human lysozyme transgenic goats | Reduced diarrhea, reduced bacterial translocation in mesenteric lymph nodes | Brundige et al. [193]; Cooper et al. [194]; Garas et al. [190] |
| K88    | Chito-oligosaccharide | Reduced diarrhea | Liu et al. [195] |
| K88    | Combination of raw potato starch and probiotic E. coli strains | Reduced diarrhea, enhanced gut microbial diversity | Krause et al. [196] |
| K88    | Probiotics: Pediococcus acidilactici, Saccharomyces cerevisiae boulardii, Bacillus subtilis | Reduced ETEC attachment to ileal mucosa, upregulated inflammatory responses in the gut | Kim et al. [142]; Daudelin et al. [197] |
| K88    | Saccharomyces cerevisiae fermented products | Enhanced appetite and ileal digesta bacteria richness, reduced ETEC adhering to the mucosa and colonic ammonia | Kiarie et al. [139, 140] |
| K88    | Probiotics: Lactobacillus plantarum CJLP243 | Enhanced growth performance, reduced diarrhea, reduced gut inflammation, enhanced gut barrier function | Lee et al. [198]; Yang et al. [199] |
| K88    | Phytogenics | Enhanced growth performance | Devi et al. [200] |
| K88    | Nucleotides | Enhanced growth performance and nutrient digestibility, reduced diarrhea | Li et al. [201] |
| F18    | Clays (smectite, zeolite, kaolinite) | Reduced diarrhea, enhanced gut integrity | Song et al. [202]; Almeida et al. [203] |
| F18    | Phytochemicals (capsicum oleoresin, garlic botanical, turmeric oleoresin) | Reduced diarrhea, enhanced gut morphology, decreased systemic and gut mucosal inflammation | Liu et al. [162, 163] |
| K88, F18 | β-glucan | Enhanced gut barrier function, reduced systemic inflammation | Stuyven et al. [138], Kim et al. [120] |

1 Modified from Liu and Ji [192].
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DOI: http://dx.doi.org/10.5772/intechopen.91219

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DOI: http://dx.doi.org/10.5772/intechopen.91219

Lactobacillus salivarius UCC118.

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