Nutritional strategies to reduce the impact of *Escherichia coli* in newly weaned pigs

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Summary

Low crude protein, added insoluble fiber, and low acid-binding capacity diets, as well as pharmacological zinc oxide (when applicable) are important nutritional strategies that can be used to maintain the gastrointestinal health of weanling pigs exposed to *Escherichia coli.* Feed additives including probiotics, exogenous enzymes, egg yolk antibodies, spray-dried plasma, clays, medium-chain fatty acids, phytogens, and antibiotics may be other options to reduce the impact of *E coli.* Management strategies such as vaccine protocols, colostrum management, barn and water line sanitation, and weaning age should be combined with nutritional interventions to minimize diarrhea caused by *E coli.*

Keywords: swine*, Escherichia coli*, wean pig, postweaning diarrhea, nutrition

Resumen - Estrategias nutricionales para reducir el impacto de la *Escherichia coli* en cerdos recién destetados

Las dietas bajas en proteína cruda, fibra añadida no soluble, y las dietas con baja capacidad de fijación de ácidos, así como el óxido de zinc farmacológico (cuando corresponda) son estrategias nutricionales importantes que pueden usarse para mantener la salud gastrointestinal de lechones destetados expuestos a la *Escherichia coli*. Los aditivos alimenticios como los probióticos, enzimas exógenas, anticuerpos de yema de huevo, plasma secado por aspersión, arcillas, ácidos grasos de cadena media, fitógenos, y antibióticos pueden ser otras opciones para reducir el impacto de la *E coli*. Las estrategias de manejo, como los protocolos de vacunación, el manejo del calostro, el saneamiento de las instalaciones y de las líneas de agua, y la edad al destete deben combinarse con intervenciones nutricionales para minimizar la diarrea causada por *E coli*.

Résumé - Stratégies nutritionnelles afin de réduire l'impact d'*Escherichia coli* chez les porcs nouvellement sevrés

Les régimes à faible teneur en protéines brutes, en fibres insolubles ajoutées et à faible capacité de fixation des acides, ainsi que l'oxyde de zinc pharmacologique (le cas échéant) sont des stratégies nutritionnelles importantes qui peuvent être utilisées pour maintenir la santé gastro-intestinale des porcelets sevrés exposés à *Escherichia coli*. Les additifs alimentaires, y compris les probiotiques, les enzymes exogènes, les anticorps de jaune d'œuf, le plasma séché par pulvérisation, les argiles, les acides gras à chaîne moyenne, les phytogènes, et les antibiotiques peuvent être d'autres options pour réduire l'impact d'*E coli*. Les stratégies de gestion telles que les protocoles de vaccination, la gestion du colostrum, l'assainissement de la bâtisse et des conduites d'eau, et l'âge de sevrage doivent être combinés à des interventions nutritionnelles pour minimiser la diarrhée causée par *E coli*.

Postweaning diarrhea (PWD) is generally characterized by loose, watery stool that occurs in the first 2 weeks post weaning.[1] The incidence of PWD is caused by a combination of different factors, including exposure to infectious pathogens such as rotavirus, *Salmonella*, or *Escherichia coli*, as well as the innate physiological and metabolic changes the young pig's gastrointestinal (GI) system undergoes around the time of weaning. In a recent study by Eriksen et al,[1] the cumulative incidence of diarrhea was estimated in 2 Danish indoor commercial production systems where no zinc oxide (ZnO) was fed. In both systems, PWD displayed a biphasic pattern with diarrhea prevalence increasing up to day 3 post weaning, and then peaking again around day 10 post weaning. Rotavirus prevalence was highest in the first 3 days after weaning, followed by *E coli* at day 10. In the United States, enterotoxigenic *E coli* (ETEC) often results in clinical illness beginning 10 to 14 days post weaning. Upon oral exposure to ETEC, pathogenesis occurs through 2 mechanisms: 1) adhesion to and colonization of the small intestinal epithelium and 2) enterotoxin secretion.[2] Long filamentous proteins called fimbriae, found on the surface of ETEC bacteria cells, adhere to specific enterocyte receptors and begin colonizing the small intestinal epithelium.[2] The 2 most common ETEC strains associated with PWD express F4 (also known as K88) and F18 fimbriae. Once colonization begins, ETEC produces enterotoxins (STa, STb, or LT) that disrupt tight junction integrity, leading to reduced nutrient absorption and increased fluid secretion into the intestinal

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lumen.[2] This results in diarrhea, reductions in growth performance, and in severe cases can lead to dehydration, electrolyte imbalances, and death. This practice tip will discuss nutritional strategies that can be used to maintain GI health of newly weaned pigs by reducing the adhesion and proliferation of *E coli* pathogens (Table 1).

Zinc and copper

To minimize pathogen-induced diarrhea in weaned pigs, pharmacological levels of zinc (2000 - 3000 ppm) from ZnO are commonly fed for the first 2 to 3 weeks after weaning.[3] The mode of action of ZnO is not well understood; however, several different mechanisms related to immune system modulation, nutrient absorption, and preservation of intestinal morphology have been identified.[4,5] While ZnO does not appear to have direct antimicrobial effects on *E coli*, it is believed that ZnO works through a multi-faceted approach to inhibit bacterial adhesion to the intestinal mucosa by blocking enterocyte receptor sites, therefore preventing the breakdown of tight junctions.[4] Copper is another mineral that can be fed at pharmacological levels to prevent pathogen induced diarrhea; however, it is less effective than ZnO. Pharmacological levels of copper (125 - 250 ppm) from copper sulfate, tribasic copper chloride, or other copper sources can be fed during early nursery in combination with high ZnO, but additive effects of the 2 minerals are not observed. Therefore, if ZnO is available to feed at elevated levels in early nursery, many producers will wait to feed elevated levels of copper until late nursery. In contrast to zinc, copper has antimicrobial effects which cause oxidative damage to bacterial cell membranes through the release of copper ions resulting in microorganism death.[5-7] In addition to the GI health benefits of zinc and copper when fed at elevated levels, growth promoting benefits have been observed.[8] Unfortunately, this practice has led to increased environmental concerns and issues surrounding bacterial resistance. The European Union has imposed regulations on the level of Zn ($\leq$ 150 ppm) and copper ($\leq$ 25 ppm) that can be included in swine diets. Canada has also started implementing feeding limits and it is expected that potential regulations will continue to be a topic of discussion within the United States. Thus, it is necessary to evaluate alternative feeding strategies to reduce the impact of enteric diseases such as *E coli*.

Alternative zinc sources

Several alternative forms of zinc have been investigated, including organic and inorganic sources and carrier mediated, nanoparticle, or microencapsulated sources.[4] Because many of these sources are more bioavailable than ZnO, alternative forms of zinc do provide an opportunity to reduce zinc inclusion levels in the diet. However, the ability of these sources to inhibit PWD have only shown intermediary effects when fed in comparison to ZnO.[4] Likewise, previous research on alternative zinc sources has only investigated levels above the inclusion limits currently imposed by the European Union. In areas such as Canada, where regulations allow zinc inclusion up to 500 ppm, there may be more opportunity to introduce other zinc sources.

Table 1: Nutritional strategies and their mode of action against *Escherichia coli*

Nutritional strategy	Primary mode of action
Zinc oxide	Inhibits pathogen adhesion by blocking enterocyte receptor sites
Copper	Pathogen death
Low crude protein	Reduces bacterial fermentation by decreasing the amount of undigested protein in the large intestine
High insoluble fiber	Inhibits pathogen adhesion by providing an alternative adhesion site
	Increases the proliferation of good bacteria to out compete pathogenic bacteria
Low acid-binding capacity diets	Reduces pathogen proliferation by decreasing gastric pH
Organic acids	Reduces pathogen proliferation by decreasing gastric pH
	Reduces pathogen proliferation by slowing down their metabolism
Probiotics	Inhibit pathogen adhesion by blocking enterocyte receptor sites
Exogenous enzymes	Inhibit pathogen adhesion by blocking enterocyte receptor sites
	Inhibit pathogen adhesion by decreasing intestinal receptor affinity for *E coli*
Egg yolk antibodies	Inhibit pathogen adhesion by neutralizing the adhesive property of *E coli* fimbriae
Spray-dried plasma	Inhibit pathogen adhesion by neutralizing the adhesive property of *E coli* fimbriae
	Inhibit pathogen adhesion by providing an alternative adhesion site for *E coli* fimbriae
Clays	Inhibit heat-labile enterotoxin absorption through the epithelial mucosa
Medium-chain fatty acids	Pathogen death
Phytogens	Pathogen death
Antibiotics	Pathogen death

Low crude protein, amino acid supplemented diets

Solid feed intake after weaning results in the secretion of hydrochloric acid in the stomach, which is essential for the breakdown and digestion of proteins.[9] However, this process may take time given that pigs experience a period of low voluntary feed intake following weaning. This ultimately results in a lower capacity to digest intact plant proteins, such as soybean meal. Specifically, when feeding a standard diet containing 21% to 23% crude protein (CP), a percentage of undigested proteins enter the large intestine leading to increased microbial fermentation and the production of diarrhetic compounds such as ammonia and amines.[10] This response is often exacerbated if pigs are exposed to pathogens, particularly when ZnO is removed from the diet. Feeding low CP diets during the first 7 to 14 days after weaning is an important strategy that can be used to decrease the amount of protein entering the large intestine for fermentation. Research consistently shows that feeding low CP diets (< 21%) decreases protein fermentation[11-13]; however, reductions in coliform producing bacteria in the ileal digesta of pigs have not accompanied this response, regardless if pigs were exposed to *E coli*.[11,14] Nevertheless, a consistent improvement in fecal dry matter has been observed when low CP diets with no ZnO are fed compared to a standard CP diet with no ZnO signifying a lesser PWD incidence.[12,15,16] In contrast to the health promoting benefits, the biggest challenge with low CP diets is the reduction in growth performance that is often observed.[15,17,18] Supplementing low CP diets with feed-grade amino acids (AA) to meet requirements can recover some losses in performance; however, reducing CP by more than 3% has resulted in conflicting responses.[19] It is likely that large CP reductions lead to AA deficiencies beyond the fifth limiting AA and may begin to limit indispensable AAs. Unfortunately, supplementation with up to 9 essential AAs or indispensable AAs, such as glycine and glutamate, still did not recover growth.[15,20] In addition to low CP diets, another way to reduce the amount of lower-digestible, plant-derived protein is to replace a portion of soybean meal with highly digestible, specialty protein sources such as spray-dried blood products, fish meal, and whey protein concentrates. These ingredients can be gradually replaced with soybean meal as the pig's ability to digest plant-derived proteins becomes more developed. This generally occurs as diets progress through phase 1 and 2 with no specialty proteins included in phase 3 diets. Feed budgets for each diet are typically fed at approximately 4, 12, and 35 lb/pig for phase 1, 2, and 3 diets, respectively.

Dietary fiber

Dietary fiber inclusion is another strategy that can be used to improve the GI health of weaned pigs. Fiber has prebiotic properties that stimulate the proliferation of "good" bacteria such as *Bifidobacterium*, *Lactobacilllus*, and *Eubacterium*, which are thought to out-compete pathogenic bacteria.[10] Furthermore, because dietary fibers are relatively indigestible, some believe they may act as alternative adhesion sites for *E coli*, therefore inhibiting *E coli* adherence to enterocytes.[21] Sources of fiber are generally divided into 2 categories: soluble and insoluble non-starch polysaccharides (NSP). Soluble fibers tend to have a higher fermentation capacity than insoluble fibers. This is of particular concern in newly weaned pigs because they have a limited capacity to ferment fiber and an accumulation of nonfermentable material in the large intestine can occur, increasing the incidence of PWD.[22] Furthermore, soluble fibers increase intestinal viscosity, which has been shown to decrease digesta passage rates leading to increased proliferation of pathogenic *E coli*.[23] Conversely, insoluble fibers have a relatively low fermentation capacity, decreased intestinal viscosity, and increased digest passage, which have been shown to increase fecal bulk.[24] Therefore, insoluble fiber sources that are high in cellulose, such as oat hulls and wheat bran are commonly used in starter diets to promote GI health.[22,25] Generally, including these fiber sources at levels to achieve an insoluble fiber inclusion of 20 to 80 g/kg is recommended.[26] As feed intake increases and their GI system becomes more developed after weaning, the pig's ability to ferment NSP increases and soluble fiber sources can be fed in place of insoluble fiber sources. Thus, early nursery pigs should be fed insoluble fiber, while late nursery pigs should be fed soluble fiber. Supplementing starter diets with 4% wheat bran has been shown to block the adhesion of *E coli* K88 to the ileal mucosa, reduce coliform counts in microbial populations, and improve fecal scores.[27,28] Feeding coarse-ground wheat bran has shown additional benefits to fine-ground wheat bran.[27] Because insoluble fibers are continuously interacting with the intestinal epithelium due to increased digesta passage rate, it is believed that coarse-ground insoluble fibers, such as wheat bran, act to "wash out" mucous-bound microbes.[26] Likewise, the larger particle size of course-ground wheat bran has a greater water holding capacity than fine-ground wheat bran, further increasing the solubility, passage rate, and fecal bulking potential of the diet.[29] Diluting the energy density of starter diets through fiber inclusion has also been shown to increase feed intake, and therefore, reduce digesta *E coli* counts and improve fecal scores.[30]

Dietary acid-binding capacity

Complete diets can alter the pigs stomach pH based on the individual ingredients fed and their capacity to bind gastric acid. Acid-binding capacity (ABC) is defined as the amount of acid required to produce a unit change in pH of a complete feed or ingredient.[31] Minerals (ie, ZnO and calcium carbonate) have a higher ABC value compared to cereal grains and bind acid more readily. A high ABC in combination with low hydrochloric acid secretion increases stomach pH leading to decreased protein digestion. Therefore, the ABC of complete diets is important to prevent enteric health challenges. The manipulation of stomach acidity can be achieved through diet formulation strategies to decrease complete feed ABC values including the reduction of minerals, such as calcium and phosphorus from calcium carbonate and sodium phosphate, or the addition of acidifiers to the diet.[31] Both have shown positive effects on pig growth performance and feed efficiency.[32-34] In addition to decreasing gastric pH, some organic acids, including butyric, formic, acetic, and propionic, also have bacteriostatic properties that act directly on gram-negative bacteria cell walls to slow down pathogen metabolism and subsequent proliferation.[35] Specifically, benzoic acid has been shown to positively influence the weight gain and fecal scores of weaned pigs inoculated with *E coli*.[36,37] In addition, the improvement in growth performance appears to remain after benzoic acid is removed from the diet[34,36] suggesting that acids may provide prolonged GI benefits. Some data also suggests that feeding coarse-ground particles improves acid production in the stomach and hindgut, therefore reducing gastric pH.[38]

Feeding diets with a low ABC has an opportunity to improve weaned pig GI health while offering protection against pathogen proliferation; however, more research is needed to make definitive recommendations on specific ABC values for each dietary phase. In addition to feed, lowering the pH of drinking water (between 4 and 6) with organic acids can improve stomach acidity and reduce *E coli* shedding in nursery pigs.[39] Periodically evaluating drinking water pH is a practical way to know when water acidification may be necessary. Likewise, cleaning barn water lines is a sanitary precaution that can be implemented to decrease the buildup of bacterial colonization in drinking water delivery systems.

Diet particle size and feed form

Based on the existing data, providing feed with particle sizes between 0.5 and 1.6 mm is recommended to optimize nutrient utilization and GI health.[38] This can be accomplished by feeding fiber-rich cereal byproducts (wheat bran) or coarse-grinding cereal grains.[40] While limited data is available regarding the influence of diet form on clinical disease associated with *E coli*, a study conducted by Longpré et al[41] reported increased cecal and colon *E coli* loads but improved growth performance in pigs fed pelleted diets compared to mash.

Feed additives

Several feed additives have been investigated for their potential to mitigate the effects of *E coli*. While many of the additives appear to be multifaceted in nature, only their primary mode of action to protect weaned pigs against enteric disease will be discussed. Modes of action can be subdivided into 2 categories: 1) pathogen adherence inhibition or 2) pathogen cell death.

Pathogen adherence inhibition

Probiotics are live microbial feed supplements that alter the microbiota of the GI tract through the direct feeding of beneficial bacteria. Therefore, it is believed that probiotic microorganisms adhere to the intestinal epithelium inhibiting the attachment of pathogenic bacteria.[42,43] Exogenous enzymes, such as xylanase, have been shown to increase the abundance of *Lactobacillus* and other microbial populations through the hydrolysis of dietary NSP which would result in a similar mode of action of probiotics against *E coli*.[44] The breakdown of NSP has also been shown to decrease digesta viscosity and improve nutrient digestibility which could aid in reducing *E coli* diarrhea. Conversely, other exogenous enzymes, such as protease or trypsin, appear to inhibit *E coli* adherence by increasing intestinal proteolytic activity. This improves protein digestion and has been shown to decrease intestinal receptor affinity for *E coli*.[45,46] Egg yolk immunoglobulin Y antibodies are another feed additive that has been shown to neutralize the adhesive property of *E coli* fimbriae preventing the attachment of *E coli* to the intestinal enterocytes,[47,48] although a relatively high dose of antibody is required.[48] Spray-dried plasma has shown similar effects to egg yolk antibodies on the growth performance and fecal consistency of pigs challenged with *E coli*.[49] Because spray-dried plasma contains immunoglobulins, this may suggest a similar mode of action. Likewise, the glycoprotein receptors found on spray-dried plasma may be responsible for binding *E coli* fimbriae, preventing their attachment to enterocyte receptors.[46,50,51] Lastly, clays act by adsorbing the heat-labile enterotoxin of *E coli* and inhibiting their absorption through the epithelial mucosa. This has been shown to alleviate PWD in *E coli*-challenged pigs.[52,53]

Pathogen cell death

Medium-chain fatty acids (MCFA) and their monoglyceride derivatives have direct antimicrobial properties that typically improve the GI health and growth performance of pigs when supplemented in the diet; although, the magnitude of response observed appears to be driven by the specific MCFA fed and inclusion level.[54] Medium-chain fatty acids act by penetrating the phospholipid membrane that surrounds bacterial and viral pathogens causing cell death.[54,55] Within the individual derivatives of MCFA, the monoglyceride has been shown to have a more potent antimicrobial effect than the fatty acids themselves.[54] Phytogens such as essential oils have also been shown to express antimicrobial properties[56] that, when fed in combination with MCFA, resulted in increased *Lactobacillus* and decreased Enterobacteriaceae fecal counts and decreased diarrhea frequency.[57] Lastly, antimicrobials such as enrofloxacin, apramycin, ceftiofur, neomycin, gentamicin, amoxicillin, and colistin can be used to treat enteric diseases.[58] However, not all these antimicrobials listed are approved for treatment of colibacillosis in the United States and care must be taken to abide by regulatory standards. Water-soluble gentamicin continues to be a common antimicrobial selection for the control and treatment of postweaning colibacillosis in the United States.[58]

Conflict of interest

None reported.

Disclaimer

Dr Gebhardt and Dr Tokach, both members of this journal's editorial board, were not involved in the editorial review of or decision to publish this article.

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